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(54) Title: DNA ENCODING ACYLCOENZYME A: CHOLESTEROL ACYLTRANSFERASE AND USES THEREOF

(57) Abstract

This invention provides an isolated nucleic acid which encodes an acylcoenzyme A: cholesterol acyltransferase II or III. Specifically, this invention provides an isolated nucleic acid which encodes a human wildtype acylcoenzyme A: cholesterol acyltransferase II or III. This invention also provides various methods for inhibiting wildtype acylcoenzyme A: cholesterol acyltransferase II or III in a subject. This invention also provides a method for identifying a chemical compound which is capable of inhibiting acylcoenzyme A: cholesterol acyltransferase II or III and a pharmaceutical compositing comprising of the chemical compound identified by the above-described method. This invention also provides a method of treating a subject who has atherosclerosis or hyperlipidemia.

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DNA ENCODING ACYLCOENZYME A: CHOLESTEROL ACYLTRANSFERASE AND USES THEREOF

This application is a Continuation-In-Part of U.S. Serial No. 08/657,620, filed May 30, 1996, the content of which is incorporated by reference into this application.

Throughout this application, various publications are referenced by Arabic numerals. Full citations for these publications may be found listed at the end of the specification. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art as known to those skilled therein.

Background of the Invention

Cholesterol or related sterols, required for the viability of eukaryotic cells, exist in the free form or as esters conjugated to fatty acids. The concentration of free sterol determines the fluidity of eukaryotic cell membranes, whereas esterified sterols cannot participate in membrane assembly. The esterification of intracellular sterol, mediated in mammals by the membrane-bound enzyme, acylcoenzyme A: cholesterol acyltransferase, is thus a critical homeostatic determinant of membrane function (1, 2). For example, cholesterol depletion of the rough endoplasmic reticulum (ER) relative to the smooth ER (3), may modulate protein translocation or membrane-associated transcriptional activators such as the Sterol Response Element Binding proteins (SREBP, 4). In addition, production of cholesterol ester (CE) by acylcoenzyme A: cholesterol acyltransferase in the rough ER may influence the transport of sterol between intracellular pools. Similar

esterification activities have been observed in other eukaryotes such as plants and yeasts (5).

Elevations in acylcoenzyme A: cholesterol acyltransferase activity perturb several pathways that contribute to hyperlipidemia and atherosclerosis. Sterol esterification modifies the activity of the low density lipoprotein (LDL) receptor and alters serum lipoprotein composition to be pro-atherogenic (6, 7). It may also be a rate limiting step in intestinal sterol absorption (8). Furthermore, CE deposition in the arterial wall is an important initial step in atherogenesis (9). The understanding of the acylcoenzyme A: cholesterol acyltransferase reaction has been hampered by the difficulty of biochemical purification and by a poor grasp of the relevant genetic determinants. A human acylcoenzyme A: cholesterol acyltransferase I gene from macrophages was identified by complementation of Chinese Hamster Ovary cell lines deficient in acylcoenzyme A: cholesterol acyltransferase activity (10) and was functionally expressed in insect cells devoid of endogenous activity (11).

Summary of the Invention

This invention provides an isolated nucleic acid which encodes an acylcoenzyme A: cholesterol acyltransferase II or an acylcoenzyme A: cholesterol acyltransferase III.

This invention also provides a vector which includes the isolated nucleic acid which encodes an acylcoenzyme A: cholesterol acyltransferase II or an acylcoenzyme A: cholesterol acyltransferase III and a host vector system which includes a vector.

This invention also provides a method of producing a polypeptide which comprises growing such host vector system of claim 14 under suitable conditions permitting production of the polypeptide and recovering the polypeptide so produced. This invention also provides a purified wildtype acylcoenzyme A: cholesterol acyltransferase II or an acylcoenzyme A: cholesterol acyltransferase III.

This invention also provides an oligonucleotide of at least 15 nucleotides capable of specifically hybridizing with a unique sequence of nucleotides present within a nucleic acid which encodes a wildtype acylcoenzyme A: cholesterol acyltransferase II or an acylcoenzyme A: cholesterol acyltransferase III without hybridizing to a nucleic acid which encodes a mutant acylcoenzyme A: cholesterol acyltransferase II or an acylcoenzyme A: cholesterol acyltransferase III. This invention also provides an oligonucleotide of at least 15 nucleotides capable of specifically hybridizing with a unique sequence of nucleotides present within the nucleic acid which encodes a mutant acylcoenzyme A: cholesterol acyltransferase II or an acylcoenzyme A: cholesterol

acyltransferase III without hybridizing to a nucleic acid which encodes a wildtype acylcoenzyme A: cholesterol acyltransferase II or an acylcoenzyme A: cholesterol acyltransferase III.

This invention also provides a method for determining whether a subject known to have an imbalance in sterol levels has the imbalance due to a defect in esterification of sterol and for treating a subject who has an imbalance in sterol levels due to a defect in esterification of sterol.

This invention also provides methods for inhibiting wildtype acylcoenzyme A: cholesterol acyltransferase II or an acylcoenzyme A: cholesterol acyltransferase III in a subject.

This invention also provides a method for identifying a chemical compound which is capable of inhibiting acylcoenzyme A: cholesterol acyltransferase II or an acylcoenzyme A: cholesterol acyltransferase III in a subject and a pharmaceutical composition comprising of the chemical compound so identified.

This invention also provides a transgenic, nonhuman mammal comprising the isolated nucleic acid which encodes acylcoenzyme A: cholesterol acyltransferase II or an acylcoenzyme A: cholesterol acyltransferase III.

Brief Description of the Figures

Abbreviations: The amino acid residues are abbreviated as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. CON: consensus sequence.

Figures 1A and 1B. Protein sequence alignments predicted from candidate genes for the human acylcoenzyme A: cholesterol acyltransferase gene I, the yeast homologs, *acylcoenzyme A: cholesterol acyltransferase-related enzyme I* and *acylcoenzyme A: cholesterol acyltransferase-related enzyme II*, and a consensus sequence of all three sequences.

Identical residues between all the sequences are in bold face. Residues of the candidate leucine zipper heptad motif are italicized. Potential transmembrane domains were identified at residues 132 to 155 and 460 to 483; 186 to 202 and 406 to 421; and 215 to 231 and 439 to 451, for human acylcoenzyme A: cholesterol acyltransferase (Sequence I.D. No.: 2), *acylcoenzyme A: cholesterol acyltransferase-related enzyme I* (Sequence I.D. No.: 4) and *acylcoenzyme A: cholesterol acyltransferase-related enzyme II* (Sequence I.D. No.: 6), respectively. The firefly luciferase signature sequences identified in human acylcoenzyme A: cholesterol acyltransferase I (10) were not conserved in the yeast genes. CON (Sequence I.D. No.: 13) denotes the consensus sequence between the sequences of human acylcoenzyme A: cholesterol acyltransferase, acylcoenzyme A:

cholesterol acyltransferase-related enzyme I and acylcoenzyme A: cholesterol acyltransferase-related enzyme II. R07932 denotes the partial sequence of another human acylcoenzyme A: cholesterol acyltransferase candidate cDNA (residues 500 to 600) (Sequence I.D. No.: 14). The asterisks indicate the residues in R07932 identical to those of the other sequences.

1A. Alignment of amino acid residues 1-362 of acylcoenzyme A: cholesterol acyltransferase-related enzyme I and the identical residues in acylcoenzyme A: cholesterol acyltransferase-related enzyme II, human acylcoenzyme A: cholesterol acyltransferase and CON.

1B. Alignment of amino acid residues 363-611 of acylcoenzyme A: cholesterol acyltransferase-related enzyme I and the identical residues in acylcoenzyme A: cholesterol acyltransferase-related enzyme II, human acylcoenzyme A: cholesterol acyltransferase and CON.

Figures 2A, 2B, 2C, 2D and 2E. Construction and analysis of acylcoenzyme A: cholesterol acyltransferase genes and deletion mutants.

2A. The *are1ΔNA* deletion. The schematic depicts a fragment from yeast chromosome III in plasmid pH3(34). Strategic restriction endonucleases are indicated (H, Hind III; B, Bam HI).

2B. The autoradiogram depicts Bam HI digested DNA from wild-type or disrupted diploid strains probed with the 2993-bp Bam-HI fragment. This produced a fragment corresponding to the wild-type acylcoenzyme A: cholesterol

acyltransferase-related enzyme I locus and a 1984-bp fragment characterizing the *are1Δ NA* allele. The diploid is heterozygous for the acylcoenzyme A: cholesterol acyltransferase-related enzyme I deletion.

2C. Reduced stringency hybridization of yeast genomic DNA with acylcoenzyme A: cholesterol acyltransferase-related enzyme I coding sequences. Genomic DNA from wild-type or ARE1/*are1ΔNA* diploids were reprobed with an Nhe I-Avr II fragment corresponding to the acylcoenzyme A: cholesterol acyltransferase-related enzyme I open reading frame ("ORF"). Hybridizations and washes were performed at 60°C in the absence of formamide.

2D. The *are2Δ* deletion. In step 1, PCR amplifying oligonucleotides, KO-5′ and KO-3′ and a *LEU2* template were used to produce the selectable yeast gene flanked at the 5′ and 3′ ends by acylcoenzyme A: cholesterol acyltransferase-related enzyme II. In step 2, this was used to direct homologous recombination at acylcoenzyme A: cholesterol acyltransferase-related enzyme II by transformation of a diploid strain and selection for leucine protrophy. In step 3, integrants to acylcoenzyme A: cholesterol acyltransferase-related enzyme II were identified by a PCR reaction using oligonucleotides flanking *ARE2* (are2-5′ and are2-3′) and a 3′ amplimer within *LEU2* (L2-3′).

2E. A 999-bp fragment identifies are2Δ, as shown in the ethidium bromide stained agarose gel. The wild-type fragment (2206-bp) is also produced

in the same reaction. Leucine prototrophic transformants with deletions of acylcoenzyme A: cholesterol acyltransferase-related enzyme II were obtained at a frequency of ~2%. M indicates the 50-2,000-bp ladder markers (Bio-Rad Laboratories).

Figures 3A and 3B. Fluorescent staining of triglyceride and sterol ester.

The cells were grown in YEPD to stationary phase, washed with deionized H_2O, and incubated with 1 μg/ml Nile Red (1 mg/ml in acetone). Fluorescent images were obtained with a BioRad MRC600 laser scanning confocal microscope (BioRad Microscience, Hercules, CA) on an inverted Zeiss Atiovert microscope (Zeiss, OberKochem, Germany) using 63X (NA1.4) Zeiss Plan-apo infinity corrected objective. Samples were illuminated with the 488nm line from an argon ion laser and the fluorescence was visualized with a 540nm dichroic mirror and 550nm long-pass emission filter. Staining of the cytoplasmic lipid droplets was sensitive to treatment with isopropanol, proving them to be lipid in nature.

3A. Wild-type cells.

3B. *are1Δ NAare2Δ* double mutant cells.

Figures 4A, 4B, 4C and 4D. Neutral lipid and sterol biosynthesis in *ARE* deletion mutants.

Strain genotypes are as described in the text; dpm/mg dry weight: disintegrations per minute per milligram of dry weight of cells.

4A. Triglyceride biosynthesis. Total lipids were

extracted from cells grown in media containing ^{3}H-oleate and analyzed by thin-layer chromatography.

4B. Sterol ester biosynthesis. Total lipids were extracted from cells grown in media containing ^{3}H-oleate and analyzed by thin-layer chromatography.

4C. Sterol ester biosynthesis in wild-type and mutant cells transformed with vector control (black box) or acylcoenzyme A: cholesterol acyltransferase-related enzyme I over-expression plasmids, YEp3-16 (increased copy number, shaded box) and pADH5-36 (transcription from the *ADH* promoter, open boxes). Cells were grown in selective media to maintain the acylcoenzyme A: cholesterol acyltransferase-related enzyme I expression plasmids. Lipids were labeled, extracted and analyzed as above.

4D. Sterol biosynthesis in acylcoenzyme A: cholesterol acyltransferase-related enzyme deletion mutants. Lipids were labeled in synthetic complete media containing [1-^{14}C] acetate, saponified and extracted with hexane and subjected to thin layer chromatography analysis. The data is representative of three separate experiments and expressed as the ratio of incorporation into sterols to incorporation into fatty acids.

Figures 5A, 5B, 5C, 5D, 5E and 5F. The nucleic acid and amino acid or predicted amino acid sequences.

5A-1 - 5A-3.

The nucleic acid sequence of human acylcoenzyme A: cholesterol acyltransferase I designated

Sequence ID No.: 1. The amino acid sequence of human acylcoenzyme A: cholesterol acyltransferase I designated Sequence ID No.: 2.

5A-1. Nucleic acid sequence of human acylcoenzyme A: cholesterol acyltransferase I from nucleic acid bases 1-1624. Amino acid sequence of human acylcoenzyme A: cholesterol acyltransferase I from amino acid residues 1-76.

5A-2. Nucleic acid sequence of human acylcoenzyme A: cholesterol acyltransferase I from nucleic acid bases 1625-2524. Amino acid sequence of human acylcoenzyme A: cholesterol acyltransferase I from amino acid residues 77-376.

5A-3. Nucleic acid sequence of human acylcoenzyme A: cholesterol acyltransferase I from nucleic acid bases 2525-3649. Amino acid sequence of human acylcoenzyme A: cholesterol acyltransferase I from amino acid residues 377-551.

5B-1 - 5B-3.

The nucleic acid sequence of yeast acylcoenzyme A: cholesterol acyltransferase-related enzyme I designated Sequence ID No.: 3. The amino acid sequence of yeast acylcoenzyme A: cholesterol acyltransferase-related enzyme I designated Sequence ID No.: 4.

5B-1. Nucleic acid sequence of acylcoenzyme A: cholesterol acyltransferase-related enzyme I from nucleic acid

bases 1-1289. Amino acid sequence of acylcoenzyme A: cholesterol acyltransferase-related enzyme I from amino acid residues 1-209.

5B-2. Nucleic acid sequence of acylcoenzyme A: cholesterol acyltransferase-related enzyme I from nucleic acid bases 1290-2114. Amino acid sequence of acylcoenzyme A: cholesterol acyltransferase-related enzyme I from amino acid residues 210-484.

5B-3. Nucleic acid sequence of acylcoenzyme A: cholesterol acyltransferase-related enzyme I from nucleic acid bases 2115-2601. Amino acid sequence of acylcoenzyme A: cholesterol acyltransferase-related enzyme I from amino acid residues 485-611.

5C-1 - 5C-3.

The nucleic acid sequence of yeast acylcoenzyme A: cholesterol acyltransferase-related enzyme II designated Sequence ID No.: 5. The amino acid sequence of yeast acylcoenzyme A: cholesterol acyltransferase-related enzyme II designated Sequence ID No.: 6.

5C-1. Nucleic acid sequence of acylcoenzyme A: cholesterol acyltransferase-related enzyme II from nucleic acid bases 1-1061. Amino acid sequence of acylcoenzyme A: cholesterol acyltransferase-related enzyme II from amino acid residues 1-238.

5C-2. Nucleic acid sequence of acylcoenzyme A: cholesterol acyltransferase-related enzyme II from nucleic acid

bases 1062-1961. Amino acid sequence of acylcoenzyme A: cholesterol acyltransferase-related enzyme II from amino acid residues 239-538.

5C-3. Nucleic acid sequence of acylcoenzyme A: cholesterol acyltransferase-related enzyme II from nucleic acid bases 1962-2421. Amino acid sequence of acylcoenzyme A: cholesterol acyltransferase-related enzyme II from amino acid residues 539-643.

5D. The nucleic acid sequence of mouse acylcoenzyme A: cholesterol acyltransferase II designated Sequence ID No.: 11. The amino acid sequence of mouse acylcoenzyme A: cholesterol acyltransferase II designated Sequence ID No.: 12.

Figure 6A. A restriction map of the expression vector YepAB-ACAT2.

Figure 6B and 6C. Expression of human macrophage ACAT in pRS426GP.

6B. The ACAT open reading frame was inserted at the *Not*I and *Sac*I sites, downstream of the promoter of the *GAL1/10* gene (GAL1/10p) as described in the text to produce pRS426-ACAT. *URA3* and Amp^r denote selectable markers for yeast and *E. coli* respectively. The yeast and bacterial origins of replication (2μm and ori, respectively) are indicated.

6C. Immunoblot of human ACAT in protein

extracts from cells transformed with pRS426-ACAT. Double mutant cells (*are1*$^-$ *are2*$^-$), transformed with pRS426-ACAT (hACAT) or with pRS426GP (vector), were induced by growth in galactose. Proteins were analyzed by immunoblotting. Equivalent amounts of protein extracts from mouse adrenal cells were loaded for comparison. Molecular weight reference markers (BioRad) are indicated (M). The arrow indicates the position of the DM10 immunoreactive product in extracts from murine adrenals. The expressed form of hACAT in yeast is of coincident mobility.

Figures 7A and 7B. Multiple human tissue Northern analysis of poly (A)+ RNAs probed with ^{32}P-labeled cDNA C1.

7A. Tissue specific expression of wildtype human acylcoenzyme A: cholesterol acyltransferase II using a wildtype acylcoenzyme A: cholesterol acyltransferase II specific probe.

7B. Tissue specific expression of wildtype human acylcoenzyme A: cholesterol acyltransferase I using a wildtype acylcoenzyme A: cholesterol acyltransferase I specific probe.

Figure 8A, 8B, 8C and 8D. Tissue specific expression of *ARGP1* and hACAT.

8A and 8B. Multiple tissue Northerns (Clontech) with indicated samples were probed with an *ARGP1* specific probe as described in the text.

8C and 8D. The same blots were also analyzed using a hACAT specific probe. The first panel is

identical to that published by Chang *et al* (8). The second panel is the same blot as in A and B, probed with the ACAT cDNA 1600 bp probe.

Figure 9. Fetal Tissue specific expression of *AGRP2*.

Multiple tissue Northerns of fetal tissue (Clontech) with indicated samples, were probed with and *AGRP2* specific probe as described in the text.

Figure 10. Cultured cell expression of *AGRP1*.

RNA samples from HepG2 and CV1 were reverse transcribed and PCR amplified as described in the text. P indicate a plasmid template control. The blank lanes represent water or no RT controls.

Figure 11. Sequence comparison of human ACAT and *AGRP1*

Figure 12. Sequence comparison of human ACAT and *AGRP2*

Figure 13. Phylogenetic Comparisons of ACAT like molecules.

The sequences shown were identified in genome databases and aligned based on protein sequence using GCG Inc software (pileup). They were subsequently arranged to their sequence conservation to determine approximate evolutionary relatedness.

Figure 14. Conserved motifs in ACAT relate gene products.

Figure 15A and 15B. Nucleotide and predicted protein sequence of ARGP1.

Figure 16. Nucleotide and predicted protein sequence of ARGP2.

Detailed Description of the Invention

Throughout this application, references to specific nucleotides are to nucleotides present on the coding strand of the nucleic acid. The following standard abbreviations are used throughout the specification to indicate specific nucleotides:

C=cytosine	A=adenosine
T=thymidine	G=guanosine

A "gene" means a nucleic acid molecule, the sequence of which includes all the information required for the normal regulated production of a particular protein, including the structural coding sequence, promoters and enhancers.

The nucleic acids or oligonucleotides of the subject invention also include nucleic acids or oligonucleotides coding for polypeptide analogs, fragments or derivatives which differ from naturally-occurring forms in terms of the identity or location of one or more amino acid residues (deletion analogs containing less than all of the residues specified for the protein, substitution analogs wherein one or more residues specified are replaced by other residues and addition analogs where in one or more amino acid residues is added to a terminal or medial portion of the polypeptides) and which share some or all properties of naturally-occurring forms. These nucleic acids or oligonucleotides include: the incorporation of codons "preferred" for expression by selected non-mammalian hosts; the provision of sites for cleavage by restriction endonuclease enzymes; and the provision of additional initial, terminal or intermediate DNA sequences that facilitate construction of readily

expressed vectors.

The nucleic acids and oligonucleotides described and claimed herein are useful for the information which they provide concerning the amino acid sequence of the polypeptide and as products for the large scale synthesis of the polypeptide by a variety of recombinant techniques. The molecule is useful for generating new cloning and expression vectors, transformed and transfected prokaryotic and eukaryotic host cells, and new and useful methods for cultured growth of such host cells capable of expression of the polypeptide and related products.

An isolated nucleic acid which encodes an acylcoenzyme A: cholesterol acyltransferase II. This isolated nucleic acid may be DNA or RNA, specifically cDNA or genomic DNA. Specifically, the isolated nucleic acid has the sequence designated Seq. I.D. No.: 7. The isolated nucleic acid encodes a human wildtype acylcoenzyme A: cholesterol acyltransferase II having substantially the same amino acid sequence as the sequence designated Seq. I.D. No.: 8. Specifically the isolated nucleic acid has the sequence designated Seq. I.D. No.: 11. The isolated nucleic acid encodes a mouse wildtype acylcoenzyme A: cholesterol acyltransferase II having substantially the same amino acid sequence as the sequence designated Seq. I.D. No.: 12. Further, the isolated nucleic acid of encodes a mutant acylcoenzyme A: cholesterol acyltransferase II.

An isolated nucleic acid which encodes an acylcoenzyme A: cholesterol acyltransferase III. This isolated nucleic acid may be DNA or RNA, specifically cDNA or genomic DNA. Specifically, the isolated nucleic acid has the sequence

as set forth in Fig. 16. The isolated nucleic acid encodes a human wildtype acylcoenzyme A: cholesterol acyltransferase III having substantially the same amino acid sequence as set forth in Fig. 16. Further, the isolated nucleic acid of encodes a mutant acylcoenzyme A: cholesterol acyltransferase III.

As used in this application, "acylcoenzyme A: cholesterol acyltransferase III" means and includes any polypeptide having acylcoenzyme A: cholesterol acyltransferase III activity and having an amino acid sequence homologous to the amino acid sequence of human acylcoenzyme A: cholesterol acyltransferase II (the sequence of which is set forth in Fig. 15). Thus, this term includes any such polypeptide whether naturally occurring and obtained by purification from natural sources or non-naturally occurring and obtained synthetically, e.g. by recombinant DNA procedures. Moreover, the term includes any such polypeptide whether its sequence is substantially the same as, or identical to the sequence of any mammalian homolog of the human polypeptide, e.g. murine, bovine, porcine, etc. homologs. Additionally, the term includes mutants or other variants of any of the foregoing which retain at least some of the enzymatic activity of nonmutants or nonvariants.

As used in this application, "acylcoenzyme A: cholesterol acyltransferase II" means and includes any polypeptide having acylcoenzyme A: cholesterol acyltransferase II activity and having an amino acid sequence homologous to the amino acid sequence of human acylcoenzyme A: cholesterol acyltransferase III (the sequence of which is set forth in Fig. 16). Thus, this term includes any such polypeptide whether naturally occurring and obtained by purification from natural sources or non-naturally

occurring and obtained synthetically, e.g. by recombinant DNA procedures. Moreover, the term includes any such polypeptide whether its sequence is substantially the same as, or identical to the sequence of any mammalian homolog of the human polypeptide, e.g. murine, bovine, porcine, etc. homologs. Additionally, the term includes mutants or other variants of any of the foregoing which retain at least some of the enzymatic activity of nonmutants or nonvariants.

The invention also encompasses DNAs and cDNAs which encode amino acid sequences which differ from those of acylcoenzyme A: cholesterol acyltransferase II, but which do not produce phenotypic changes.

The invention also encompasses DNAs and cDNAs which encode amino acid sequences which differ from those of acylcoenzyme A: cholesterol acyltransferase III, but which do not produce phenotypic changes.

The nucleic acid of the subject invention also include nucleic acids that encode for polypeptide analogs, fragments or derivatives which differ from naturally-occurring forms in terms of the identity or location of one or more amino acid residues (including deletion analogs containing less than all of the residues specified for the protein, substitution analogs wherein one or more residues specified are replaced by other residues and addition analogs wherein one or more amino acid residues is added to a terminal or medial portion of the polypeptides) and which share some or all properties of the naturally-occurring forms.

The polypeptide of the subject invention also includes analogs, fragments or derivatives which differ from

naturally-occurring forms, but having acylcoenzyme A: cholesterol acyltransferase activity.

This invention also provides a vector comprising an isolated nucleic acid encoding acylcoenzyme A: cholesterol acyltransferase II or III. The isolated nucleic acid of the vectors is operatively linked to a promoter of RNA transcription which maybe, or is identical to, a bacterial, yeast, insect or mammalian promoter. The vector may be a plasmid, cosmid, yeast artificial chromosome (YAC), bacteriophage or eukaryotic viral DNA. Specifically, this invention provides a vector designated YepAB-ACAT2 (Figure 6).

Further other numerous vector backbones known in the art as useful for expressing proteins may be employed. Such vectors include but are not limited to: adenovirus, simian virus 40 (SV40), cytomegalovirus (CMV), mouse mammary tumor virus (MMTV), Moloney murine leukemia virus, murine sarcoma virus, and Rous sarcoma virus, DNA delivery systems, i.e liposomes, and expression plasmid delivery systems.

This invention also provides a vector system for the production of a polypeptide which comprises the vector in a suitable host. Suitable host includes a cell which includes, but is not limited, prokaryotic or eukaryotic cells, e.g. bacterial cells (including gram positive cells), yeast cells, fungal cells, insect cells and animal cells.

Suitable animal cells include, but are not limited to, HeLa cells, Cos cells, CV1 cells and various primary mammalian cells. Numerous mammalian cells may be used as hosts, including, but not limited to, the mouse

fibroblast cell NIH 3T3, CHO cells, Ltk$^-$ cells, etc. Expression plasmids such as that described supra may be used to transfect mammalian cells by methods well known in the art such as calcium phosphate precipitation, electroporation.

This invention also provides a method for producing a polypeptide (e.g. acylcoenzyme A: cholesterol acyltransferase) which comprises growing a host vector system under suitable conditions permitting production of the polypeptide and recovering the polypeptide so produced. Methods of recovering polypeptides produced in such host vector systems are well-known in the art and typically include steps involving cell lysis, solubilization and chromatography.

This invention also provides a method of obtaining a polypeptide in purified form which comprises: (a) introducing a vector, as described above, into a suitable host cell; (b) culturing the resulting cell so as to produce the polypeptide; (c) recovering the polypeptide produced in step (b); and (d) purifying the polypeptide so recovered. As discussed above the vector may include a plasmid, cosmid, yeast artificial chromosome, bacteriophage or eukaryotic viral DNA. Also, the host cell may be a bacterial cell (including gram positive cells), yeast cell, fungal cell, insect cell or animal cell. Suitable animals cells include, but are not limited to HeLa cells, Cos Cells, CV1 cells and various primary mammalian cells. Culturing methods useful for permitting transformed or transfected host cells to produce polypeptides are well known in the art as are the methods for recovering polypeptides from such cells and for purifying them.

Using the aforementioned method, this invention also provides a purified wildtype acylcoenzyme A: cholesterol acyltransferase II or III and a purified mutant acylcoenzyme A: cholesterol acyltransferase II or III.

This invention also provides an oligonucleotide of at least 15 nucleotides capable of specifically hybridizing with a unique sequence of nucleotides present within a nucleic acid which encodes a wildtype acylcoenzyme A: cholesterol acyltransferase II or III without hybridizing to a nucleic acid which encodes a mutant acylcoenzyme A: cholesterol acyltransferase II or III. Further, this invention also provides an oligonucleotide of at least 15 nucleotides capable of specifically hybridizing with a unique sequence of nucleotides present within the nucleic acid which encodes a mutant acylcoenzyme A: cholesterol acyltransferase II or III without hybridizing to a nucleic acid which encodes a wildtype acylcoenzyme A: cholesterol acyltransferase II or III. These oligonucleotide DNA or RNA. Such oligonucleotides may be used in accordance with well known standard methods for known purposes, for example, to detect the presence in a sample of DNA which will hybridize thereto.

The oligonucleotides include, but are not limited to, oligonucleotides that hybridize to mRNA encoding acylcoenzyme A: cholesterol acyltransferase II or III so as to prevent translation of the protein.

This invention also provides a nucleic acid having a sequence complementary to the sequence of the isolated nucleic acid which encodes acylcoenzyme A: cholesterol acyltransferase II or III.

This invention also provides a method for determining

whether a subject known to have an imbalance in sterol levels has the imbalance due to a defect in esterification of sterol which comprises (a) obtaining from the subject an appropriate sample containing a mixture of all of the subject's nucleic acids; and (b) determining whether any nucleic acid in the sample from step (a) is, or is derived from, a nucleic acid which encodes a mutant acylcoenzyme A: cholesterol acyltransferase so as to thereby determine whether the subject's imbalance in sterol levels is due to a defect in esterification of sterol. The determination step (b) may comprises: (I) contacting the sample of step (a) with the isolated nucleic acid which encodes acylcoenzyme A: cholesterol acyltransferase II or III or the oligonucleotide of at least 15 nucleotides capable of specifically hybridizing with a unique sequence of nucleotides present within a nucleic acid which encodes a wildtype acylcoenzyme A: cholesterol acyltransferase II or III without hybridizing to a nucleic acid which encodes a mutant acylcoenzyme A: cholesterol acyltransferase II or III under conditions permitting binding of any nucleic acid in the sample which is, or is derived from, a nucleic acid which encodes a mutant acylcoenzyme A: cholesterol acyltransferase to the nucleic acid or oligonucleotide so as to form a complex; (ii) isolating the complex so formed; and (iii) identifying the nucleic acid in the isolated complex so as to thereby determine whether any nucleic acid in the sample contains a nucleic acid which is, or is derived from, a nucleic acid which encodes a mutant acylcoenzyme A: cholesterol acyltransferase II or III. In this method, both the isolation of any complex formed are effected using standard methods well known in the art.

In order to facilitate identification of the nucleic acid

from step (a) the isolated nucleic acid or the oligonucleotide is labeled with a detectable marker. The detectable marker may be a radioactive isotope, a fluorophore or an enzyme. In additions, the nucleic acid sample may be bound to a solid matrix before performing step (I).

This invention also provides a method for treating a subject who has an imbalance in sterol levels due to a defect in esterification of sterol which comprises introducing an isolated nucleic acid which encodes a wildtype acylcoenzyme A: cholesterol acyltransferase II or III into the subject under conditions such that the nucleic acid expresses a wildtype acylcoenzyme A: cholesterol acyltransferase II or III, so as to thereby treat the subject.

This invention also provides a method for inhibiting wildtype acylcoenzyme A: cholesterol acyltransferase II or III in a subject which comprises transforming appropriate cells from the subject with a vector which expresses the nucleic acid complementary to the isolated nucleic acid which encodes a wildtype acylcoenzyme A: cholesterol acyltransferase II or III, and introducing the transformed cells into the subject so as to thereby inhibit wildtype acylcoenzyme A: cholesterol acyltransferase II or III. Further, in a preferred embodiment, the nucleic acid is capable of specifically hybridizing to a mRNA molecule encoding acylcoenzyme A: cholesterol acyltransferase II or III so as to prevent translation of the mRNA molecule.

This invention also provides a method for inhibiting the wildtype acylcoenzyme A: cholesterol acyltransferase II or III in a subject which comprises introducing an

oligonucleotide of at least 15 nucleotides capable of specifically hybridizing with a unique sequence of nucleotides present within a nucleic acid which encodes a wildtype acylcoenzyme A: cholesterol acyltransferase II or III without hybridizing to a nucleic acid which encodes a mutant acylcoenzyme A: cholesterol acyltransferase II or III into the subject so as to thereby inhibit the wildtype acylcoenzyme A: cholesterol acyltransferase II or III. The oligonucleotide is capable of specifically hybridizing to a mRNA molecule encoding acylcoenzyme A: cholesterol acyltransferase II or III so as to prevent translation of the mRNA molecule.

This invention also provides for a method for identifying a chemical compound which is capable of inhibiting acylcoenzyme A: cholesterol acyltransferase II or III in a subject which comprises (a) contacting a wildtype acylcoenzyme A: cholesterol acyltransferase II or III with the chemical compound under conditions permitting binding between the acylcoenzyme and the chemical compound (b) detecting specific binding of the chemical compound to the acylcoenzyme; and © determining whether the chemical compound inhibits the activity of the coenzyme so as to identify a chemical compound which is capable of inhibiting acylcoenzyme A: cholesterol acyltransferase II or III in a subject.

This invention also provides method for differentially inhibiting one acylcoenzyme A: cholesterol acyltransferase but not others using the above methods. In an embodiment, only acylcoenzyme A: cholesterol acyltransferase I is inhibited. In another embodiment only acylcoenzyme A: cholesterol acyltransferase II (ARGP1) is inhibited. In an another embodiment only acylcoenzyme A: cholesterol acyltransferase III (ARGP2)

is inhibited. Alternatively, two of the acylcoenzyme A: cholesterol acyltransferases may be inhibited. This invention further provides pharmaceutical compositions which will differentially inhibit one or more acylcoenzyme A: cholesterol acyltransferases.

This invention also provides for a pharmaceutical composition comprising the chemical compound identified by the above-described method in an amount effective to inhibit acylcoenzyme A: cholesterol acyltransferase II or III in a subject and a pharmaceutically effective carrier.

This invention also provides a method of treating a subject who has atherosclerosis comprising the above-described pharmaceutical composition. A method of treating a subject who has hyperlipidemia comprising the above-described pharmaceutical composition.

This invention also provides a transgenic, nonhuman mammal comprising the isolated nucleic acid which encodes acylcoenzyme A: cholesterol acyltransferase II or III. The mammal includes, but is not limited to, a mouse, bovine, cat or dog.

This invention is illustrated in the Experimental Details section which follows. These sections are set forth to aid in an understanding of the invention but are not intended to, and should not be construed to, limit in any way the invention as set forth in the claims which follow thereafter.

Experimental Details

First Series of Experiments

Example 1:

Materials and Methods:

Transformation of yeast was performed with lithium acetate (15) by amino-acid prototrophy selection. A diploid strain (5051) was constructed between two isogenic derivatives of W303 (16); W1346-3C (*MAT*a, *ade2-1, can1-100, his3-11, 15, leu2-3, 112, trp1-1, ura3-1*) and W1134-2C (*MAT*a, *can1-100, his3-11, 15, leu2-3, 112, trp1-1, ura3-1, met14DHpaI-SalI*). Growth on complete (YEPD) or synthetic medium, sporulation and dissection was performed as described (17).

Competent cells of *Escherichia coli* strain DH5a (Gibco-BRL) and DNA modifying enzymes (Promega) were used according to the manufacturers instructions. pH3(34), from L.A. Grivell, was digested with Nhe I, blunt-ended with Klenow sequences, and digested with Avr II to liberate a 1614-bp fragment. An Xba I, Sma I fragment of pJH-H1 encoding the *HIS3* gene was then inserted at these sites in the vector backbone to produce the *are1ΔNA* allele. This construct was digested with Bsa I to liberate a 3821-bp fragment which was then transformed into strain 5051. Disruption of *ARE1* was confirmed by Southern blot analysis.

Radioactive probes of acylcoenzyme A: cholesterol acyltransferase-related enzyme I were prepared by random priming (Pharmacia) with ^{32}P-dCTP. Genomic DNA (18) was transferred to Hybond membranes (Amersham) and hybridized in the absence of formamide at 65° or 60°C (19).

A shotgun library of cosmid 14-21 from chromosome XIV (Peter Philippsen, Biozentrum Basel) was constructed using the nebulizing technique (20). The DNA was nebulized (90 seconds, 2 bars), size fractionated, treated with DNA polymerase I (Klenow fragment) and T4 DNA polymerase and blunt-end ligated into pTZ18R (Pharmazia, Germany). Nucleotide sequencing was performed by dideoxy-chain-termination with digoxigenin-labeled reverse primer and Sequenase (United States Biochemical). The reactions were analyzed on the GATC 1500 direct blotting electrophoresis system (GATC GmbH, Germany) using the Boehringer-Mannheim Dig-development protocol. Sequences were aligned by SeqMan (DNA Star Inc.). Database searching was performed with BLAST (21) and GCG Inc. software (22). The DNA sequence of the *acylcoenzyme A: cholesterol acyltransferase-related enzyme I* and *acylcoenzyme A: cholesterol acyltransferase-related enzyme II* genes are deposited at GenBank (P25628 and U51790, respectively).

KO-5 and KO-3' primers (GAGGGGACGAAAATTAGCCGCTATTAATTCTGGTATTGCCACCTAGACAAGAAG TAAACAGACACAGATGcaagagttcgaatctcttagc (Sequence ID No.: 15)and CTATAAAGATTTAATAGCTCCACAGAACAGTTGCAGGATGCCTTAGGGT CGActacgtcgtaaggccgtttctgac (Sequence ID No.: 16), respectively; lower case corresponds to the *LEU2* gene) were used in a PCR with the *LEU2* gene as a template to produce the selectable yeast gene flanked by *acylcoenzyme A: cholesterol acyltransferase-related enzyme II* gene sequences (23). This was used to transform a derivative of yeast strain 5051, heterozygous for the *are1ΔNA* allele. To identify integrants at the *acylcoenzyme A: cholesterol acyltransferase-related enzyme II* locus, a PCR was performed on genomic DNA from these strains using

are2-5' (CATTGCAGTTACACGTGAATGC) (Sequence ID No.: 17), are2-3': (TAGCTCCACAGAACAGTTGCAGG) (Sequence ID No.: 18) and a 3' amplimer corresponding to the *LEU2* gene (L2-3' CTCTGACAACAACGAAGTCAG) (Sequence ID No.: 19).

1-2 units at an absorbance of 6000nm of cells were incubated in YEPD or defined media containing 1 μCi/ml ^{3}H-oleate in tyloxapol/ethanol (1:1) for 16 hours. Total lipids were prepared by hexane extraction (25) and analysed by thin layer chromatography on DC-plastikfolien kieselgel 60 plates (E-Merck, Germany). The plate was developed in hexane, diethyl ether and acetic acid (70:30:1) and stained with iodine vapor. Incorporation of label into triglyceride and ergosterol ester was ascertained following scintillation counting and normalization to a ^{14}C-cholesterol internal standard and the dry weight of the cells.

To overexpress the acylcoenzyme A: cholesterol acyltransferase-related enzyme I gene by copy number under the control of its own promoter in YEp3-16, a 2354 bp Cla I fragment from pH3(34), encompassing the entire acylcoenzyme A: cholesterol acyltransferase-related enzyme I gene, was made blunt-ended with Klenow DNA polymerase I and introduced into the Sma I site of YEp352. To constitutively overexpress acylcoenzyme A: cholesterol acyltransferase-related enzyme I from the ADH promoter in pADH5-36, a 2290 bp Nar I fragment of pH3(34), starting 70 bp 5' to the ORF was blunt-ended with Klenow and ligated to Klenow-treated, Eco RI digested, pDC-ADH (a derivative of pS5) (26). Increased expression of the acylcoenzyme A: cholesterol acyltransferase-related enzyme I transcripts, relative to a wild-type cell, was confirmed by northern blot

analysis.

The incorporation of [1-^{14}C] acetate into saponified lipids was assessed as a measurement of sterol synthesis. Approximately 2 OD_{600} units of cells were incubated with 20 μCi [1-^{14}C] acetate in 2 ml defined media at 30°C for 3 hours and subjected to lipid saponification, hexane extraction and TLC chromatography (29). The incorporation of counts into total sterols were assessed following scintillation counting. To normalize the estimate of sterol biosynthesis to incorporation of acetate into the fatty acid pool, the aqueous lysate remaining after hexane extraction was acidified with concentrated HCl and re-extracted with hexane (30).

Experimental Discussion

To use yeast genetics to study sterol esterification, the human acylcoenzyme A: cholesterol acyltransferase sequence was used to search for homologous yeast genes and subsequently to identify an additional human isoform (Figures 1A and 1B). Acylcoenzyme A: cholesterol acyltransferase related enzyme I, an 1830-bp open reading frame (ORF) on yeast chromosome III, encodes a 610-residue protein with 23% identity and 49% similarity to human acylcoenzyme A: cholesterol acyltransferaseI (Figures 1A and 1B). The yeast and human proteins possess leucine zipper motifs that could mediate protein-protein interactions (esterification is probably performed by a multimeric complex) (12), and possess at least two predicted transmembrane domains that may mediate the membrane association of the acylcoenzyme A: cholesterol acyltransferase reaction (13, 14).

To define the role of acylcoenzyme A: cholesterol

acyltransferase-related enzyme I in sterol esterification, the deletion mutant, *are1ΔNA*, was generated by homologous recombination (15, 16, 17) (Fig. 2A). In a diploid strain, a 1614-bp segment of one acylcoenzyme A: cholesterol acyltransferase-related enzyme I allele was replaced with the *HIS3* gene and confirmed by Southern hybridization (Fig. 2B). Analysis of mutant and wild-type haploid progeny from this diploid indicated no differences in growth rates or incorporation of ^{3}H-oleate into ergosterol ester.

The lack of a defect in sterol esterification in *are1ΔNA* strains could result from alternate esterification activities. Reduced stringency hybridization of yeast genomic DNA with the acylcoenzyme A: cholesterol acyltransferase-related enzyme I coding sequence as a probe indicated that additional homologous sequences were present (18, 19). A Bam HI digestion of genomic DNA produced the predicted 2.9-kb acylcoenzyme A: cholesterol acyltransferase-related enzyme I fragment and a ~6.0-kb hybridizing fragment (Fig. 2C). Contour clamped homogeneous electric field electrophoretic analysis of yeast chromosomes suggested the latter sequence was localized to chomosome X or XIV. On the basis of homology to acylcoenzyme A: cholesterol acyltransferase-related enzyme I, this gene, designated acylcoenzyme A: cholesterol acyltransferase-related enzyme II, encodes a second yeast homolog to human acylcoenzyme A: cholesterol acyltransferaseI (Figures 1A and 1B). The genomic sequence (20, 21, 22) encompassing acylcoenzyme A: cholesterol acyltransferase-related enzyme II on chromosome XIV predicts a 5997-bp Bam HI fragment and a 1929-bp ORF, which translates into a 643-residue polypeptide. The yeast acylcoenzyme A: cholesterol

acyltransferase related enzymes genes are 61% and 49% identical at the DNA and predicted protein levels, respectively. Arelp, Are2p and the human acylcoenzyme A: cholesterol acyltransferaseI protein are most related at the COOH-terminal region (42% identity over a 90-residue sequence) (Figures 1A and 1B).

To assess the contribution of Are2p to sterol esterification, one copy of the acylcoenzyme A: cholesterol acyltransferase-related enzyme II coding sequence was deleted from the genome of an *ARE1/are1ΔNA* heterozygous diploid by a polymerase chain reaction approach (23) (Fig. 2D). Haploid progeny representing the single *are1ΔNA* and *are2Δ* deletions and the *are1Δ NAare2Δ* double mutant were obtained. To ascertain the effect of deletion of acylcoenzyme A: cholesterol acytransferase-related enzymes genes upon cytoplasmic lipid storage, the neutral lipid components (triglyceride and sterol ester) of the yeast cells were detected by fluorescence microscopy after staining with Nile Red (24). In wild-type cells, cytoplasmic fluorescent droplets accumulated in stationary phase cultures (Fig. 3A). No differences in *are* single mutants were detected. However, the number of droplets observed in *are1ΔNAare2Δ* double mutants, was one-third to that in wild-type strains (Fig. 3B; over multiple fields, 5.57 ± 2.73 vs. 16.73 ± 4.6 droplets/cell, $P<0.05$).

The wild-type and *are* mutant cells were analyzed for the incorporation of ^{3}H-oleate into sterol ester (25) (Fig. 4B). No significant differences in triglyceride biosynthesis were detected. In contrast to normal sterol ester biosynthesis observed in *are1ΔNA* mutants, deficiencies in sterol esterification were apparent in

both *are2Δ* and *are1ΔNAare2Δ* mutants. These were detected by iodine vapor staining of thin layer chromatographs of total yeast lipids in addition to the oleate incorporation assays. Sterol ester levels of *are2Δ* single mutants were reduced to less than 26% of wild-type suggesting the acylcoenzyme A: cholesterol acyltransferase-related enzyme II isoform to confer the majority of acyltransferase activity. The *are1ΔNAare2Δ* double mutant was almost totally deficient in sterol esterification (less than 1% of wild-type levels). In confirmation of the critical role of Are proteins in sterol esterification, microsomes from double mutant yeast cells lacked acylcoenzyme A: cholesterol acyltransferase activity when assayed *in vitro*.

To confirm that the protein encoded by an acylcoenzyme A: cholesterol acyltransferase-related enzymes ORF was sufficient for sterol esterification, the acylcoenzyme A: cholesterol acyltransferase-related enzyme I coding sequence was over-expressed in vectors with increased copy number (YEp3-16) or elevated transcription (the alcohol dehydrogenase promoter in pADH5-36) (26). There were no detectable changes in triglyceride or phospholipid biosynthesis resulting from acylcoenzyme A: cholesterol acyltransferase-related enzyme I over-expression. In *are2Δ* or *are1ΔNAare2Δ* double mutants, acylcoenzyme A: cholesterol acyltransferase-related enzyme I over-expression complemented the sterol esterification defect (Fig. 4C). In wild-type and *are1DNA* single mutants, the high level expression of acylcoenzyme A: cholesterol acyltransferase-related enzyme I did not elevate sterol ester synthesis above untransformed controls. This suggests that either substrates are limiting in acylcoenzyme A: cholesterol

acyltransferase-related enzymes strains or that the enzyme is post-translationally regulated as in mammalian cells (27).

An accumulation of unesterified sterol in cell membranes would likely be deleterious (28). However, despite the major changes in sterol esterification conferred by the *are* mutants, we did not detect any reduction in growth rates. The established role of sterol esterification in the storage of sterol suggests that an inability to esterify sterol could lead to homeostatic changes in sterol biosynthesis. This relationship might account for the viability of the mutants. Total lipids, labelled by the incorporation of [1-^{14}C] acetate into exponentially growing cells (29, 30), were saponified and extracted. The *are1 Δ Naare2Δ* double mutants had a two to three-fold lower level of sterol biosynthesis than wild-type cells, although no changes were observed in the single mutants (Fig. 4D). In fact, free sterol concentrations were roughly equivalent in all cells. Feedback regulation of sterol biosynthesis by acylcoenzyme A: cholesterol acyltransferase activity has been observed in mammalian cells (31) and may be a common mechanism that maintains intracellular sterol at non-toxic concentrations.

The involvement of multiple gene families in sterol homeostasis is common in mammalian and yeast cells, for example, the LDL receptor related protein and scavenger receptor gene families, the SREBP family, and 3-hydroxy-3-methyl-glutanyl-CoA reductase) (4, 32, 33, 34). This apparent redundancy of function has clear physiological consequences as evidenced by deletion of any one of the family members. The observation here of two yeast genes for sterol esterification provoked the hypothesis of

similar redundancy for this reaction in humans. To this end, a consensus of the yeast acylcoenzyme A: cholesterol acyltransferase-related enzymes and human acylcoenzyme A: cholesterol acyltransferaseI sequences was used to identify an additional cDNA with significant identity (47%) to human acylcoenzyme A: cholesterol acyltransferaseI and the yeast proteins (Figure 1B, Genbank accession # R07932).

Sterol homeostasis is a complex event under subtle regulatory controls, one component of which is sterol esterification. The demonstration here of multiple yeast and human acylcoenzyme A: cholesterol acyltransferase isoforms raises the possibility that *in vivo*, the enzymes exhibit alternate substrate preferences. The analysis of esterification reactions in yeast is likely to impact the understanding of sterol homeostasis and atherosclerosis in humans.

Example 2:

Tissue specific expression of acylcoenyme A: cholesterol acyltransferase II was analyzed by Northern blot RNA hybridization of RNA obtained from the described tissues. Using the same materials and procedures of Chang, et al. (10), the specific expression of acylcoenyme A: cholesterol acyltransferase II in liver and muscle is documents, in contrast to similar experiments using the previously known acylcoenyme A: cholesterol acyltransferase I (10) (Figures 7A and 7B). Acylcoenyme A: cholesterol acyltransferase II was also detected and specifically expressed in adrenal, thyroid and testicular tissues.

Example 3:

After determining the consensus sequence between the two yeast gene and the previously known human acylcoenzyme A: cholesterol acyltransferase, the consensus sequence was compared to sequences deposited in Genbank. The clones containing the sequences that showed similarity to the consensus sequence were ordered from the I.M.A.G.E. Consortium, affiliated with Research Genetics, Inc., 2130 Memorial Parkway S.W. Huntsville, Alabama 35801. Clones deposited with the I.M.A.G.E. consortium are publicly available upon request. A particular clone, Genbank ID clone No. Z39933 was chosen. This clone contains a cDNA fragment whose sequence encodes human acylcoenyme A: cholesterol acyltransferase II. The fragment was cut out with restrictions enzymes Bgl II and Not I. The resulting fragment was introduced into the yeast expression vector pRS426 at Bgl II and Not I sites downstream of the yeast promoter (GAL1/GA110) which is regulated by carbon sources. The resultant vector was designated YepAB-ACAT2 (Figure 6).

Example 4:

Antisense RNA technology can be used to create mice, or mouse or human cell lines incapable of translating acylcoenzyme A: cholesterol acyltransferase II RNA into protein. Standard methods may be used to create an antisense oligonucleotide to the human homolog of acylcoenzyme A: cholesterol acyltransferase II These methods are well known in the art (36).

Specifically, part or all of a wildtype acylcoenzyme A: cholesterol acyltransferase II is ligated adjacent to a mammalian promoter in the opposite orientation. The

promoter and other replicatory mechanisms inside the cell will transcribe a human homolog of acylcoenzyme A: cholesterol acyltransferase II encoding, nonsense strand. This strand will bind with the coding mRNA which is normally synthesized to form a complex. Due to the formation of this complex, the antisense strand prevents the translation of the coding mRNA into protein.

Further, one skilled in the art can synthesize an oligonucleotide *in vitro* which is capable of binding the mRNA that encodes a human homolog of acylcoenzyme A: cholesterol acyltransferase II so as to inhibit the translation of the mRNA into protein. The oligonucleotides can then be introduced into the subject using a pharmaceutically acceptable carrier. Methods of synthesizing naturally and non-naturally occurring oligonucleotides which are capable of inhibiting the translation of the mRNA into protein are well known in the art. Also, means of transfecting an organism with such oligonucleotides are well known in the field.

Example 5:

Mice can be made with an alteration in their genome, specifically at the acylcoenyme A: cholesterol acyltransferase II gene site. Standard methods may be used to alter the genome. These methods are well known in the art (37, 38).

One such process to achieve this goal involves disrupting the wildtype mouse homolog of acylcoenyme A: cholesterol acyltransferase II *in vitro*, then introducing the altered gene into mouse embryonal stem cells in such a way as to taret integration into the corresponding genomic region. This process can be performed such that both copies of

the wildtype acylcoenyme A: cholesterol acyltransferase II are replaced by the altered, knock-out version. These modified cells can be introduced into blastocysts which will be allowed to develop into chimeric adults. Mice bearing the altered acylcoenyme A: cholesterol acyltransferase II gene will be mated to each other to generate homozygous mutant acylcoenyme A: cholesterol acyltransferase II animals.

Further, one can breed two mice who are heterozygous for mutant acylcoenzyme A: cholesterol acyltransferase II. From their progeny, one skilled in the art could select the progeny who are homozygous for mutant acylcoenzyme A: cholesterol acyltransferase II. Breeding and selecting such progeny are well known in the art.

References of the First Series of Experiments

1. T.-Y. Chang and G.M. Doolittle, in *The Enzymes*, P. Boyer, Eds. (Academic Press, New York, 1983), vol. XVI, pg. 523.
2. K. E. Bloch, *CRC Crit. Rev. Biochem.* **14**, 47 (1983).
3. M. P. Reinhart, J. T. Billheimer, J. R. Faust, J. L. Gaylor, *J. Biol. Chem.* **262**, 9649 (1987).
4. X. Wang *et al.*, *Cell* **77**, 53 (1994).
5. J. T. Billheimer and P. J. Gillies, in *Advances in Cholesterol Research* M. Esfahani, J. B. Swaney, Eds. (Telford Press, Philadelphia, 1992).
6. T. P. Carr, J. S. Parks, L. L. Rudel, *Arterio. and Thromb.* **12**, 1275 (1992).
7. S. C. Rumsey, N. F. Galeano, B. Lipschitz, R. J. Deckelbaum, *J. Biol. Chem.* **270**, 10008 (1995).
8. T. Shiratori and D. S. Goodman, *Biochem. Biophys. Acta* **106**, 625 (1965).
9. S. S. Katz, G. G. Shipley, D. M. Small, *J. Clin. Inv.* **58**, 200 (1976).
10. C. C. Y. Chang, H. Y. Huh, K. M. Cadigan, T. Y. Chang, *J. Biol. Chem.* **268**, 20747 (1993).
11. D. Cheng, C. C. Y. Chang, X. M. Qu, T. Y. Chang, *J. Biol. Chem.* **270**, 685 (1994).
12. S. K. Erickson, S. R. Lear, M. J. McCreery, *J. Lipid Res.* **35**, 763 (1994).
13. D. S. Goodman, D. Deykin and T. Shiratori, *J. Biol. Chem.* **239**, 1335 (1964).
14. G. M. Doolittle and T. Y. Chang, *Biochem.* **21**, 674 (1982).
15. H. Ito, Y. Fukuda, K. Murata, A. Kimura, *J. Bacteriol.* **153**, 163 (1983)
16. B. J. Thomas and R. Rothstein, *Cell* **56**, 619 (1989).
17. F. Sherman and J. Hicks, in *Methods in Enzymology* C. Guthrie, G. R. Fink, Eds. (Academic Press, 1991),

vol. 194, pp. 21.

18. C. S. Hoffman and F. Winston, *Gene* **57**, 267 (1987).

19. F. M. Ausubel *et al.*, *Current Protocols in Molecular Biology* (John Wiley & Sons, New York, 1987), vol. 1.

20. C. M. Okpodu *et al.*, *BioTechniques* **16**, 154 (1994).

21. S. F. Altschul *et al.*, *J. Mol. Biol.* **215**, 403 (1990).

22. J. Devereux, P. Haeberli, O. Smithies, *Nucl Acids Res* **12**, 387 (1984)

23. A. Baudin, O. Ozier-Kalogeropoulos, A. Denouel, C. Cullin, *Nuc. Acids Res.* **21**, 3329 (1993).

24. P. Greenspan, E. P. Mayer, S. D. Fowler, *J. Cell Biol.* **100**, 965 (1985).

25. L. W. Parks, C. D. Bottema, R. J. Rodriguez, T. A. Lewis, *Methods Enzymol* **111**, 333 (1985).

26. S. L. Sturley *et al.*, *J. Biol. Chem.* **269**, 21670 (1994).

27. C. C. Chang *et al.*, *J. Biol. Chem.* **270**, 29532 (1995).

28. G. J. Warner *et al.*, *J. Biol. Chem.* **270**, 5772 (1995).

29. R. Y. Hampton and J. Rine, *J. Cell Biol.* **125**, 299 (1994).

30. D. Dimster-Denk, M. K. Thorsness, J. Rhine, *Mol. Biol. Cell* **5**, 655 (1994).

31. I. Tabas, D. A. Weiland, A. R. Tall, *J. Biol. Chem.* **261**, 3147 (1986).

32. M. Krieger and J. Herz, *Annu. Rev. Biochem* **63**, 601 (1994).

33. X. Wang *et al.*, *Cell* **77**, 53 (1994).

34. M. E. Basson, M. Thorsness, J. Rine, *Proc. Natl. Acad. Sci. USA* **83**, 5563 (1986).

35. S. L. Thompson, R. Burrows, R. J. Laub, S. K. Krisans, *J. Biol. Chem.* **262**, 17420 (1987).

36. Griffin, E.F. and H. Harris (1992) *J. Cell Science* **102**: 799-805.

37. Joyner, A.L. and F. Guillemot (1994) *Current Opinion in Neurobiology* **4**: 37-42.

38. Duyao, M.P., et al. (1995) *Science* **269**: 407-410.

Second Series of Experiments

The efficient regulation of intracellular sterol levels is required for cell viability by all eukaryotic organisms. When this regulation is aberrant in cells of the arterial wall, disease states such as atherosclerosis ensue. A critical component of this homeostasis is intracellular sterol esterification reaction, mediated by the enzyme, acyl coenzyme A-cholesterol acyltransferase (ACAT). In the model eukaryote, yeast, this laboratory has demonstrated that sterol esterification is mediated by a two gene family (Yang et al., Science, 1996, 272:1353). The existence in human cells of two additional genes encoding ACAT related enzymes are demonstrated. These protein are termed ACAT related gene products (ARGP) 1 and 2, also known as acylcoenzyme A: cholesterol acyltransferase II and acylcoenzyme A: cholesterol acyltransferase III respectively. The ARGPs exhibit marked sequence conservation to the human ACAT sequence (hACAT) originally identified by Chang and colleagues. ARGP1 is expressed at high levels in intestine and liver in contrast to the expression of hACAT which is of low abundance in these tissues. The observation that knock-out mutant mice deficient in the murine homolog if hACAT retain sterol esterification activity in liver and intestine (Meiner et al., PNAS, 1996, 93:14041), suggests that ARGP1 is a candidate for sterol esterification in these tissues. The expression of ARGP2, by contrast, seems to be restricted to the fetal liver, suggesting it to have a role in lipid metabolism during development. Analysis of genome databases indicates that ACAT-like gene families are a common occurrence in multiple organisms. It is hypothesize that multiple enzymes for sterol esterification will provide flexibility in response to differing sterol and fatty acid substrates encountered by different tissues. This further suggests specific roles

for these enzymes in lipoprotein production, lipid homeostasis, and disease progression.

The regulation of membrane sterol levels is required for cell viability by all eukaryotic organisms. When this regulation is aberrant in human cells, disease states such as atherosclerosis (excessive accumulation of cellular esterified cholesterol in cells of the arterial wall, reviewed in (1-4)), Niemann Pick C (inability to store sterol correctly, resulting in lysosomal lipidosis, (5)) or Wollmann's disease (a defect in sterol ester hydrolysis, (6)) ensue. A critical component of this homeostasis is the intracellular neutralization of sterol by an esterification reaction between the C_3-OH group of cholesterol and fatty acyl-coenzyme A. This reaction is performed in mammalian cells by the enzyme acyl coenzyme A-cholesterol acyltransferase (ACAT). Since the process of sterol esterification converts sterol into a cytoplasmic storage form, it is critical to all eukaryote, including the microorganism Saccharomyces cerevisiae (budding yeast). Analysis if sterol homeostasis in this model organism has the advantage that molecular genetics, particularly since the completion of the yeast genome sequencing project, is powerful and relatively straightforward. Taking advantage of this, it is demonstrated that sterol esterification in yeast is mediated by a two gene family (7), neither of which is essential for life. These genes (ARE1 and ARE2; encoding ACAT Related Enzymes 1 and 2, respectively) are both capable of independently esterifying sterol, although in terms of contribution to the sterol ester mass of the cell, Are1 is a minor isoform relative to Are2. The genes are structurally and functionally analogous to the ACAT sequence isolated originally from macrophages by Chang and colleagues (8). They share approximately 23% identity at the protein level and expression of the human macrophage ACAT cDNA in yeast are

double deletion mutants results in esterification of sterol (9).

A critical test of the role of the ACAT gene product in cholesterol homeostasis and atherosclerosis was initiated by Farese and colleagues, by the production of "knock-outs" at the Acact locus corresponding to the mouse homolog of hACAT (10). The fidelity of the mutation was confirmed by sequencing of cDNA from the disrupted allele and by the failure to detect immunoreactive protein in Acact$^{-/-}$ cell extracts. The animals were healthy and fertile and had residual, but significant, sterol esterification activity in fibroblasts and macrophages. Cholesterol ester levels and ACAT activity in the adrenals were also severely reduced. Conversely, Acact$^{-/-}$ livers contained significant levels of cholesterol ester, and esterification activity was not altered. Furthermore, sterol absorption in the intestine, a process that probably requires esterification, was unaffected by the gene disruption. These observations strongly suggest that as in yeast, there are multiple genes for the ACAT reaction in mammalian cells, probably with tissue specific expression patterns.

Interestingly, despite the clear origin of the yeast gene family by gene duplication, the ARE proteins have diverged such that the majority of sequence conservation is in the COOH-terminal domain of the protein. This is presumably the critical region of the molecule, since it is also conserved in the human protein. Using this region as a database probe, R07932 (11) was identified, a partially sequenced cDNA entry in the database of expressed sequence tags (best); R07932 exhibits significant similarity to the ACATs particularly over the COOH-terminal region. Taken together; the "founder" sequence, the observations in yeast of a two gene family for sterol esterification, and the tissue-specific

expression patterns of enzyme activity in Acact$^{-/-}$knock-out mice, suggest that there are multiple genes for this reaction in all eukaryote. It is reported here the isolation and characterization of cDNAs from two human loci that encode ACAT Related Gene Products (ARGP). ARGP1 is represented multiple times in the best, including R07932, and is expressed ubiquitously with the highest levels occurring in the liver, intestine and adrenal gland. By contrast, sequences identical to ARGP2 in the databases are infrequent, consistent with the observation of an essentially embryonic pattern of expression. Analysis of genome databases indicates that gene families that conserve these motifs are a common occurrence in multiple organism.

Materials and Methods.

Database searching for ACAT related sequences. A sequence corresponding to the strongest region of protein conservation between the human macrophage ACAT and yeast ARE sequences was used to identify protein sequences predicted to be encoded by entries in the best using the tblastn software (NCBLI). The DNA sequences thus arising were used to detect additional clones in any available database, that demonstrated overlaps of nucleotide sequence identity. Databases searched included; best, the non-redundant GENBANK, and the confidential database held at The Institute of Genome Research (TIGR). Overlaps between these sequences were detected using the sequence alignment programs, "lineup" and "pileup" from GCG Inc (Madison, WI). A consensus sequence was then generated. Escherichia coli clones with the largest inserts corresponding to these sequences (see table 1) were obtained from the I.M.A.G.E. consortium and resequenced from both ends using commercial primers, T3 and T7, or internal primers derived from a consensus. Nucleotide sequencing was performed at the Columbia University Combined Center core facility using an Applied

Biosystems fluorescent sequencing machine.

Table 1: Entries of human ACAT related gene products in the products in the data base of expressed sequence tags.

Gene	Clone ID (IMAGE)	GENEBANK ID	Insert size (bp)	Comments
ARGP1	200587	R99213	620	
		R99214		
	55218	c-IMF11	1800	chimera
		Z43867		
		Z33993		
	1881180	H45923	1000	
		H45924		
	78614	M79086	300	
	153836	R48474	800	
		R48475		
	106260	T35085	800	
ARGP2	128921	R10272	680	
		R10273		
	213176	N75438	540	
	245265	H76642	300	

Isolation and sequencing analysis of full length cDNA clones of ARGP1 and ARGP2. Since in no instance were any of the database clones full length for either ARGP1 or ARGP2, additional clones with intact 5'-ends are described. Several strategies were chosen using a consensus nucleotide sequence derived from the sequencing of the best clones designed and synthesized 3-end, gene specific primers and used a PCR based, rapid amplification of cDNA ends (RACE) to derive 5'-RACE reaction products from a human liver/spleen Marathon library (Clontech®). Similar strategy was used to derive PCR products from a human fetal brain library generously

provided by Bento Soares (Columbia University). In some instances, a nested PCT reaction was performed using internal gene specific primers and library adaptors. Finally primer extension cDNA products were identified from mRNA extracted from human intestine (a kind gift of P. Dawson). Amplification products of the predicted size were confirmed as gene specific, using southern hybridization to sequences predicted to be at the 3'-end of these products. The products were isolated from agarose gels using Geneclean and subcloned into TA variants of pBluescript (Stratagene®) vectors of klenow/kinase treated and blunt end ligated to pGEM2 (Promega®). Positive clones were identified by colony hybridization or by PCR amplifications using an internal ARGP specific primer. Clones with the largest inserts were sequenced to obtain novel sequence and where necessary, this process was reiterated with ARGP 5' specific primers derived from the new sequence.

Tissue specific expression of hACAT and ARGps. Fragments of the best clones R99213 and R10273 corresponding to ARGP1 and ARGP2, respectively were derived by digestion with EcoRI and NotI, and purified from agarose gels with Geneclean. A 1.6 kbp fragment corresponding to the human ACAT cDNA identified by Chang et al was used as a probe for the expression of this gene. Radiolabelled probes were generated by random priming (Pharmacia®) in the presence of ^{32}-P dCTP and used to probe Multiple Tissue Northerns (MTN, Clontech®) of human samples. Hybridizations were performed, according to the manufacturers instructions, using ExpressHyb rapid hybridization solution for 1 hour at 78°C, followed by washed in 2xSSC at 55°C and 0.1xSSC, 0.5%SDS at 50°C.

Cell culture expression of ARGPs. To facilitate quantitation of mRNA from the ARGP genes, a reverse-transcriptase PCR (RT-PCR) approach was devised to

analyze expression in a variety of human (HeoG2, THP-1 macrophages) and rodent (J774 macrophages) and simian (CV1 kidney cells). Where possible, primers were designed to be conserved between rodents and humans (as described below, the mouse sequence homolog to ARGP1) has been identified. Alternatively, PCR conditions were optimized to permit moderate mismatches. The ARGP amplification primers were designed to be gene specific (i.e. to regions not conserve within the family) and to produce distinct size products.

Experimental Results and Discussion

The approach that the region of strongest conservation between the yeast ARE proteins and hACAT would be critical to the function of any sterol esterification enzymes was taken. A region of conservation (consensus; LN---E---FGDR-FY GDWWN, single letter amino-acid code) that is invariant over the three proteins was chosen and a series of entries derived from gene sequencing projects identified. In addition to sequences from Caenorhabditis. elegans, Schizosacharomuces pombe, Drosophila melanogater and Arabidopis thaliens, several entries in the best of human cDNAs that suggested an independent gene encoding an ACAT like protein were observed. Using the nucleotide sequence to this clone, a second homologous but distinct entry was identified. These proteins are termed, ACAT Related Gene Products (ARGP) 1 (acylcoenzyme A: cholesterol acyltransferase II) and 2 (acylcoenzyme A: cholesterol acyltransferase III). The sequence identified by Chang et al (8) will be referred to as hACAT, hereon. A limited protein sequence to a founder clone (R07932) to ARGP1 has been presented previously (11). The entries in the best that define these two genes, including their insert sizes are described in table 1. As is evident, the majority of inserts (with the exception of a chimeric clone ZA3867) are less than 1Kbp. The northern and sequence analysis

presented indicated them to be incomplete clones. However, they clearly define two distinct genes of strong similarity to the ACAT sequence, with the majority of predicted protein conservation at the COOH-terminal region. As described below certain motifs considered critical to sterol esterification are conserved. To identify the role of these genes in the reaction, full length ARGP clones were sought and their patterns identified.

ARGP1, a ubiquitously expressed member of the ACAT gene family. To establish the profile of expression of ARGP1, probed multiple tissue northerns of human mRNA was probed, using a fragment close to the 3' end of the gene. Although this region displays the maximum conservation at the protein level in this gene family, the genes are sufficiently divergent at the DNA level to be able to design gene specific hybridization probes. The ARGP1 sequence is expressed at abundant levels in may tissues with the exception of lung and kidney. The majority of tissues express a 2.0kb message but, some tissues (e.g. adrenal, small intestine, thymus) also express a 2.4kb mRNA at varying levels. The same northerns were hybridized with a probe to the human macrophage ACAT sequence. As described by others(8,12,13), the hACAT sequence detects 4 messages of approximately 3.0, 4.0, 4.7 and 7.4Kb. Upon comparison of the two hybridization results, an overlapping but occasionally differential expression pattern was observed. Adrenal tissues express the highest levels of both hACAT and ARGP1 message. By this analysis, hACAT messages are rare in liver and intestine in contrast to ARGP1 which is highly expressed in these tissues. Conversely, ARGP1 was poorly expressed in kidney, lung and placenta although hACAT mRNA was easily detected. This tissue specific expression suggests that ARGP1 is an ideal candidate for sterol esterification in tissues such as liver and intestine,

which retain sterol esterification activity in ACAT k/o mice (10).

ARGP2, an embryonic isoform of the ACAT gene family. Efforts to identify a transcript from ARGP2 in adult tissues were unsuccessful. Therefore embryonic tissue samples were chosen to investigate since the original founder clone was derived from a fetal liver library. A multiple tissue northern of mRNA from human embryonic brain, liver, kidney, and lung, were probed with and ARGP2 specific, COOH-terminal probe. As shown in figure 9, a single message of ~2.2kb was identified only in embryonic liver tissues, suggesting a high degree of tissue and developmental specificity to the expression of this gene product.

Expression of ARGP1 in cell culture models. To develop a system in which to test the effect of reaction substrates on the esterification reaction performed by the ARGP enzymes. The expression of these genes in several tissue specific were examined, cell culture models. As shown in figure 10, ARGP1 is clearly expressed in liver (HepG2) and Kidney (CV-1) cell lines. The latter result is somewhat in contrast to the northern blot on human tissue samples. This most likely reflects the sensitivity of the RT-PCR approach compared to filter hybridization and suggests that ARGP1 is probably expressed in most tissues. Alternatively it may represent species difference (simian vs. human) or more interestingly the differentiation status of the cells under study. In data not shown here, ARGP1 was also clearly expressed in human and mouse macrophage models (THP-1 and J774 cells).

Sequence characteristics of ARGP1 and ARGP2. By a combination of 5'-RACE and primer extension additional sequence to cDNA s for ARGP1 and ARGP2 (Figs. 11 and 12)

have been identified. The ARGP1 sequence predicts a 407 amino-acid protein with approximately 27% identity and 52% similarity to the hACAT protein (Fig. 4). Interestingly, as it was observed for the yeast ARE proteins, the strongest conservation exists at the COOH-terminus of the molecules, to the extent that the NH-2-terminal 50% of all these proteins is essentially unrelated sequence. This pattern also persists at the DNA level (not shown). Identification of the genomic sequence to these cDNAs will establish whether this remarkable divergence arises by exon shuffling of common sequences. Alternatively, convergent evolution of domains with conserved functions in sterol esterification or related processes, may have resulted in the generation of these families. Since the level of DNA conservation between ARGP1 and hACAT is quite low (37% identity), the latter possibility seems likely. The conserved regions are discussed in the context of multiple ACAT like sequences below. The ARGP1 sequence predicts a protein of approximately 47kDa with multiple transmembrane domains in similar positions to those predicted in hACAT. This strongly suggests a membrane location for ARGP1 as would be predicted for a sterol esterification enzyme.

ARGP2 displays a significantly higher level of amino acid conservation with hACAT than does ARGP1. Over the sequence shown (Fig. 12), the protein is 59% identical and 79% similar to human ACAT. Over the same region ARGP1 is only conserved at the level of 32% identity. This striking identity is maintained at the DNA level (62% identity) and may suggest that ARGP2 is more closely analogous to hACAT in both its mechanism of action and its origin, than is ARGP1. As for ARGP1, certain hallmark sequences are retained in ARGP2 (see below). The ARGP2 predicted protein also possesses several predicted transmembrane domains. One entry to the best for ARGP2 has also been allocated an STS (sequence tagged

site) at the Whitehead Institute, (entry # WI-11660) and has thus been mapped to human chromosome 12.

Sterol esterification enzymes evolve as gene families in multiple organisms. Using the hACAT and AGRP nucleotide sequences as probes of multiple databases, we sought to establish whether the observation of gene families of ACAT related enzymes in yeast and humans was a common occurrence in other organisms. In general this is the case (Fig. 13). Sequences from the genome of C. elegans, D. melanogastor and S.pombe, have been identified that are distinct from each other, within an organism, and exhibit approximately 25% identity at the predicted protein level. As for all the ACAT-like proteins, the maximum conservation is observed at the COOH-terminal region, with many of the apparently critical motifs described below, being maintained. As would be anticipated the mouse cDNA for ARGP1 exhibits approximately 85% identity with its human homolog.

Sequence conservation between ARGPs and ACAT in multiple organisms. As described above, these sequences are ubiquitous. This conservation, across and within organisms, facilitates the identification of critical domains of esterification enzymes (Fig. 14). Interestingly, there is no sequence similarity between any ACAT-like molecule and lecithin cholesterol acyltransferase (LCAT), despite the shared utilization of cholesterol. For the hACAT sequence and its murine homologs, a similarity to "signature' motifs of enzymes involved in acyl adenylation reactions was reported (8, 12). However, these sequences are unlikely to be critical, since they are not conserved in any homolog from any other organism. By contrast, there are regions of strong conservation between these molecules which may be critical to function. In the esterification defective, SRD4 mutant CHO cell line, the expressed but

defective ACAT allele encodes a single amino-acid substitution of leucine$_{265}$ lies in a conserved domain of human, rodent and yeast ACAT. Interestingly, this motif in ARGP1 is more degenerate, although the serine is conserved, the flanking sequence is conservatively replaced by similar residues. The ACAT reaction is probably mediated by a multimeric complex, as shown by radiation inactivation experiments (15). Accordingly, the yeast and human sequences all possess "leucine zipper" multimerization motifs. ARGP1 and ARGP2 lack a classical multimerization motif. Although protein phosphorylation as a mode of ACAT regulation has been refuted (16), a very strong region of conservation (consensus over 7 sequences; LN---E---FGDR-FYGDWWN, single letter amino-acid code) predicts a tyrosine kinase consensus motif for phosphorylation. ARGP2 and ARGP1 are no exception to this. In particular the aspartic acid-tryptophan-tryptophan-asparagine (DWWN) sequence appears to be invariant (with the exception of S.pombe, where it is AWWN) and may represent an active site for the esterification reaction. These regions if conservation are targets for mutagenesis and in preliminary experiments appear critical to the activity of the ACAT and ARE enzymes (17).

Why ACAT gene families? The role, if any, of these ACAT sequence homologs in sterol homeostasis is unclear. Since mouse macrophage ACAT is not critical to sterol esterification in the liver and intestine, it is possible that the additional enzymes evolved to recognize alternate substrates and thus promote sterol absorption in the intestine or production of lipoproteins by the liver (39). Future experiments will be directed to complete the molecular characterization of these genes and test these hypotheses.

References of the Second Series of Experiments

1. Liscum, L., and Underwood, K.W. (1995) J. Biol. Chem. **270**(26), 15443-15446

2. Chang, T.Y., Chang, C.C.Y., and Cadigan, K.M. (1994) Trends Cadiovascul. Med. **4**, 223-230

3. Tabas, I. (1995) Curr. Op. in Lipidol. **6**(5), 260-268

4. Libby, P., and Clinton, S.K. (1993) Curr. Opin. Lipidol. **4**, 3355-363

5. Pentchev, P.G., Brady, R.O., Blanchette-Mackie, E.J., Vanier, M.T., Carstea, E.D., Parker, C.C., Goldin, E., and Roff, C.F. (1994) Biochim. Biophys. Acta **1225**, 235-243

6. Goldstein, J.L., Dana, S.E., Faust, J.R., Beaudet, A.L., and Brown, M.S. (1975) Journal of Biological Chemistry **250**(21), 8487-95

7. Yang, H., Bard, M., Bruner, D.A., Gleeson, A., Deckelbaum, R.J., Aljinovic, G., Pohl, T., Rothstein, R., and Sturley, S.L. (1995) Circulation **In Press**

8. Chang, C.C.Y., Huh, H.Y., Cadigan, K.M., and Chang, T.Y. (1993) J. Biol. Chem. **268**(28), 20747-20755

9. Yang, H., Cromley, D., Wang, H., Billheimer, J.T., and Sturley, S.L. (1997) J. Biol. Chem. **272**, 3980-3985

10. Meiner, V.M., Cases, S., Myers, H., Snade, E.R., Bellosta, S., Schambelan, M., Pitas, R.E., McGuire, J., Herz, J., and Farese, R.V. (1996) Proc. Natl. Acad. Sci. USA **93**, 14041-14046

11. Yang, H., Bard, M., Bruner, D.A., Gleeson, A., Deckelbaum, R.J., Aljinovic, G., Pohl, T., Rothstein, R., and Sturley, S.L. (1996) Science **272**, 1353-1356

12. Uelman, P.J., Oka, K., Sullivan, M., Chang, C.C.Y., Chang, T.Y., and Chan, L. (1995) J. Biol. Chem. **270**, 26192-26201

13. Pape, M.E., Schultz, P.A., Rea, T.J., DeMattos, R.B., Kieft, K., Bisgaier, C.L., Newton, R.S., and

Krause, B.R. (1995) Journal of Lipid Research **36**(4), 823-38

14. Cao, G., Goldstein, J.L., and Brown, M.S. (1996) Journal of Biological Chemistry **271**(24), 14642-8

15. Erickson, S.K., Lear, S.R., and McCreery, M.J. (1994) J. Lipid Res. **35**, 763-769

16. Corton, J.M., and Hardie, D.G. (1992) European Journal of Biochemistry **204**(1), 203-8

17. Guo, Z., Yang, H., and Sturley, S.L. (1996) Circulation **94**, I-35

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(I) APPLICANT: Stephen L. Sturley

(ii) TITLE OF INVENTION: DNA ENCODING ACYLCOENZYME A: CHOLESTEROL ACYLTRANSFERASE 11 AND USES THEREOF

(iii) NUMBER OF SEQUENCES: 19

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
© OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

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(B) FILING DATE: Herewith
© CLASSIFICATION:

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(2) INFORMATION FOR SEQ ID NO:1:

(I) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3649 base pairs
(B) TYPE: nucleic acid
© STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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ACAGTGAATT TCTGATTTCA CTCAGGGTGA TAAATCAGAC TCTTGGGGAA GCGGGTGGTG 240

GCTCTGGACA GCAGCAGGAA TGGGGATCCA GTTAGCAACA AATCCATGGA CCTATGACAG 300

GCTGAAAGCC ACCCCTTCTC CATCTTTGGG AGGTTGCCAA TGTCTGATTT AACACTATCC 360

AATGAATGAT CATTGAAAGT AAAAAATAAC TATCAACTAG CAGAAAATAT AAATGGTAAG 420

CATTAGCACA TATTTCACAT GTTTATATTT GGCTCTCAGA TTGACCTATA AAACAAAGTC 480

TGGGAAATTC TATATGATCC TGAAAAAATG ATACGCTGGT CTGGATGGTA GAATAAGTTG 540

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TAATTTTTTT GAAAAAAGTT CAATGTTCAG TTTTCCTTAGT TTTTACCTT 3660

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 550 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Amino Acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Ser Leu Glu Thr Pro Ser Asn Gly Arg Ile Asp Ile Lys Gln Leu Ile
35 40 45

Ala Lys Lys Ile Lys Leu Thr Ala Glu Ala Glu Glu Leu Lys Pro Phe
50 55 60

Phe Met Lys Glu Val Gly Ser His Phe Asp Asp Phe Val Thr Asn Leu
65 70 75 80

Ile Glu Lys Ser Ala Ser Leu Asp Asn Gly Gly Cys Ala Leu Thr Thr
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Phe Ser Val Leu Glu Gly Glu Lys Asn Asn His Arg Ala Lys Asp Leu
100 105 110

Arg Ala Pro Pro Glu Gln Gly Lys Ile Phe Ile Ala Arg Arg Ser Leu
115 120 125

Leu Asp Glu Leu Leu Glu Val Asp His Ile Arg Thr Ile Tyr His Met
130 135 140

Phe Ile Ala Leu Leu Ile Leu Phe Ile Leu Ser Thr Leu Val Val Asp
145 150 155 160

Tyr Ile Asp Glu Gly Arg Leu Val Leu Glu Phe Ser Leu Leu Ser Tyr
165 170 175

Ala Phe Gly Lys Phe Pro Thr Val Val Trp Thr Trp Trp Ile Met Phe
180 185 190

Leu Ser Thr Phe Ser Val Pro Tyr Phe Leu Phe Gln His Trp Arg Thr
195 200 205

Gly Tyr Ser Lys Ser Ser His Pro Leu Ile Arg Ser Leu Phe His Gly
210 215 220

Phe Leu Phe Met Ile Phe Gln Ile Gly Val Leu Gly Phe Gly Pro Thr
225 230 235 240

Tyr Val Val Leu Ala Tyr Thr Leu Pro Pro Ala Ser Arg Phe Ile Ile
245 250 255

Ile Phe Glu Gln Ile Arg Phe Val Met Lys Ala His Ser Phe Val Arg
260 265 270

Glu Asn Val Pro Arg Val Leu Asn Ser Ala Lys Glu Lys Ser Ser Thr
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Val Pro Ile Pro Thr Val Asn Gln Tyr Leu Tyr Phe Leu Phe Ala Pro
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Thr Leu Ile Tyr Arg Asp Ser Tyr Pro Arg Asn Pro Thr Val Arg Trp
305 310 315 320

Gly Tyr Val Ala Met Lys Phe Ala Gln Val Phe Gly Cys Phe Phe Tyr
325 330 335

Val Tyr Tyr Ile Phe Glu Arg Leu Cys Ala Pro Leu Phe Arg Asn Ile
340 345 350

Lys Gln Glu Pro Phe Ser Ala Arg Val Leu Val Leu Cys Val Phe Asn
355 360 365

Ser Ile Leu Pro Gly Val Leu Ile Leu Phe Leu Thr Phe Phe Ala Phe
370 375 380

Leu His Cys Trp Leu Asn Ala Phe Ala Glu Met Leu Arg Phe Gly Asp
385 390 395 400

Arg Met Phe Tyr Lys Asp Trp Trp Asn Ser Thr Ser Tyr Ser Asn Tyr
405 410 415

Tyr Arg Thr Trp Asn Val Val Val His Asp Trp Leu Tyr Tyr Tyr Ala
420 425 430

Tyr Lys Asp Phe Leu Trp Phe Phe Ser Lys Arg Phe Lys Ser Ala Ala
435 440 445

Met Leu Ala Val Phe Ala Val Ser Ala Val Val His Glu Tyr Ala Leu
450 455 460

Ala Val Cys Leu Ser Phe Phe Tyr Pro Val Leu Phe Val Leu Phe Met
465 470 475 480

Phe Phe Gly Met Ala Phe Asn Phe Ile Val Asn Asp Ser Arg Lys Lys
485 490 495

Pro Ile Trp Asn Val Leu Met Trp Thr Ser Leu Phe Leu Gly Asn Gly
500 505 510

Val Leu Leu Cys Phe Tyr Ser Gln Glu Trp Tyr Ala Arg Arg His Cys
515 520 525

Pro Leu Lys Asn Pro Thr Phe Leu Asp Tyr Val Arg Pro Arg Ser Trp
530 535 540

Thr Cys Arg Tyr Val Phe
545 550

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2601 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GCCCTCCAGC TCTCTACTAA GACCGGTCGC AAGCATGCTG GGCGATATAT CCAAACCACA 60

CCACACATGG TCTCCCTCCT GCGTCAAAAT CTCCCCAGAC AGTCCGGACC CGCACCCGAT 120

ATCCAGAATG AAACTGCACG GCTGCAGATT CAAAAGCTCC AACGCCCTCA GCGTCATCTT 180

CGCCTGGATA TGCTGCACTC TGGTCGAACC CGTGTACTTG TGTGCTTCGC TATCATTATA 240

GAAAATCTCC GGTGGTGCCA ACTCCTCAGG ACGTGACATT ATTTCTTCTC TGATATATTT 300

CCTGTGTTTC CGTACCGCAC CTTTTTAGCA CTACTTTTTT ACTATGCTCT TCTTCTTCTG 360

CTTCTTCTGC TTTTTTCCTC TTTATCACAC TATGTATGTG CTGCTCATCT CTTCTTTTTA 420

TCGATAAAAT TGAAAAATGT GAGATGGTGT AGAGTGAAAA AAAAAAAAAA ATCTGGCTTG 480

GCCATCAAAT ACCCGGCCGT GGTTGGACTC GTTTAGCGAA CAATAGCACC CAGCAGACCC 540

TGGCAACATG CGGATGATAT AAGAAGGACG AGCGTGGTGG AGGAAAGGGG CGCCATTGGC 600

ACACTCACGC AGGTGGTTGT TCAGCACGGC TTGCAGCAAG AGCGCCAAAA CAGATTGCAA 660

GAATGACGGA GACTAAGGAT TTGTTGCAAG ACGAAGAGTT TCTTAAGATC CGCAGACTCA 720

ATTCCGCAGA AGCCAACAAA CGGCATTCGG TCACGTACGA TAACGTGATC CTGCCACAGG 780

AGTCCATGGA GGTTTCGCCA CGGTCGTCTA CCACGTCGCT GGTGGAGCCA GTGGAGTCGA 840

CTGAAGGAGT GGAGTCGACT GAGGCGGAAC GTGTGGCAGG GAAGCAGGAG CAGGAGGAGG 900

AGTACCCTGT GGACGCCCAC ATGCAAAAGT ACCTTTCACA CCTGAAGAGC AAGTCTCGGT 960

CGAGGTTCCA CCGAAAGGAT GCTAGCAAGT ATGTGTCGTT TTTTGGGGAC GTGAGTTTTG 1020

ATCCTCGCCC CACGCTCCTG GACAGCGCCA TCAACGTGCC CTTCCAGACG ACTTTCAAAG 1080

GTCCGGTGCT GGAGAAACAG CTCAAAAATT TACAGTTGAC AAAGACCAAG ACCAAGGCCA 1140

CGGTGAAGAC TACGGTGAAG ACTACGGAGA AAACGGACAA GGCAGATGCC CCCCCAGGAG 1200

AAAAACTGGA GTCGAACTTT TCAGGGATCT ACGTGTTCGC ATGGATGTTC TTGGGCTGGA 1260

TAGCCATCAG GTGCTGCACA GATTACTATG CGTCGTACGG CAGTGCATGG AATAAGCTGG 1320

AAATCGTGCA GTACATGACA ACGGACTTGT TCACGATCGC AATGTTGGAC TTGGCAATGT 1380

TCCTGTGCAC TTTCTTCGTG GTTTTCGTGC ACTGGCTGGT GAAAAAGCGG ATCATCAACT 1440

GGAAGTGGAC TGGGTTCGTT GCAGTGAGCA TCTTCGAGTT GGCTTTCATC CCCGTGACGT 1500

TCCCCATTTA CGTCTACTAC TTTGATTTCA ACTGGGTCAC GAGAATCTTC CTGTTCCTGC 1560

ACTCCGTGGT GTTTGTTATG AAGAGCCACT CGTTTGCCTT TTACAACGGG TATCTTTGGG 1620

ACATAAAGCA GGAACTCGAG TACTCTTCCA AACAGTTGCA AAAATACAAG GAATCTTTGT 1680

CCCCAGAGAC CCGCGAGATT CTGCAAAAAA GTTGCGACTT TTGCCTTTTC GAATTGAACT 1740

ACCAGACCAA GGATAACGAC TTCCCCAACA ACATCAGTTG CAGCAATTTC TTCATGTTCT 1800

GTTTGTTCCC CGTCCTCGTG TACCAGATCA ACTACCCAAG AACGTCGCGC ATCAGATGGA 1860

GGTATGTGTT GGAGAAGGTG TGCGCCATCA TTGGCACCAT CTTCCTCATG ATGGTCACGG 1920

CACAGTTCTT CATGCACCCG GTGGCCATGC GCTGTATCCA GTTCCACAAC ACGCCCACCT 1980

TCGGCGGCTG GATCCCCGCC ACGCAAGAGT GGTTCCACCT GCTCTTCGAC ATGATTCCGG 2040

GCTTCACTGT TCTGTACATG CTCACGTTTT ACATGATATG GGACGCTTTA TTGAATTGCG 2100

TGGCGGAGTT GACCAGGTTT GCGGACAGAT ATTTCTACGG CGACTGGTGG AATTGCGTTT 2160

CGTTTGAAGA GTTTAGCAGA ATCTGGAACG TCCCCGTTCA CAAATTTTTA CTAAGACACG 2220

TGTACCACAG CTCCATGGGC GCATTGCATT TGAGCAAGAG CCAAGCTACA TTATTTACTT 2280

TTTTCTTGAG TGCCGTGTTC CACGAAATGG CCATGTTCGC CATTTTCAGA AGGGTTAGAG 2340

GATATCTGTT CATGTTCCAA CTGTCGCAGT TTGTGTGGAC TGCTTTGAGC AACACCAAGT 2400

TTCTACCGGC AAGACCGCAG TTGTCCAACG TTGTCTTTTC GTTTGGTGTC TGTTCAGGGC 2460

CCAGTATCAT TATGACGTTG TACCTGACCT TATGAACTGC CACCATACCA CGTGTGTCCC 2520

TCGCAAGCCC TTGATAGATA TACAATAGGG AATGGGCGTC CGTCCACCGT GGTCAAAGAC 2580

AGGGGCAAAG AGCTCCTAGG T

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 610 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Amino Acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Thr Glu Thr Lys Asp Leu Leu Gln Asp Glu Glu Phe Leu Lys Ile
1 5 10 15

Arg Arg Leu Asn Ser Ala Glu Ala Asn Lys Arg His Ser Val Thr Tyr
20 25 30

Asp Asn Val Ile Leu Pro Gln Glu Ser Met Glu Val Ser Pro Arg Ser
35 40 45

Ser Thr Thr Ser Leu Val Glu Pro Val Glu Ser Thr Glu Gly Val Glu
50 55 60

Ser Thr Glu Ala Glu Arg Val Ala Gly Lys Gln Glu Gln Glu Glu Glu
65 70 75 80

Tyr Pro Val Asp Ala His Met Gln Lys Tyr Leu Ser His Leu Lys Ser
85 90 95

Lys Ser Arg Ser Arg Phe His Arg Lys Asp Ala Ser Lys Tyr Val Ser
100 105 110

Phe Phe Gly Asp Val Ser Phe Asp Pro Arg Pro Thr Leu Leu Asp Ser
115 120 125

```
Ala Ile Asn Val Pro Phe Gln Thr Thr Phe Lys Gly Pro Val Leu Glu
    130                 135                 140

Lys Gln Leu Lys Asn Leu Gln Leu Thr Lys Thr Lys Thr Lys Ala Thr
145                 150                 155                 160

Val Lys Thr Thr Val Lys Thr Thr Glu Lys Thr Asp Lys Ala Asp Ala
                165                 170                 175

Pro Pro Gly Glu Lys Leu Glu Ser Asn Phe Ser Gly Ile Tyr Val Phe
            180                 185                 190

Ala Trp Met Phe Leu Gly Trp Ile Ala Ile Arg Cys Cys Thr Asp Tyr
        195                 200                 205

Tyr Ala Ser Tyr Gly Ser Ala Trp Asn Lys Leu Glu Ile Val Gln Tyr
    210                 215                 220

Met Thr Thr Asp Leu Phe Thr Ile Ala Met Leu Asp Leu Ala Met Phe
225                 230                 235                 240

Leu Cys Thr Phe Phe Val Val Phe Val His Trp Leu Val Lys Lys Arg
                245                 250                 255

Ile Ile Asn Trp Lys Trp Thr Gly Phe Val Ala Val Ser Ile Phe Glu
            260                 265                 270

Leu Ala Phe Ile Pro Val Thr Phe Pro Ile Tyr Val Tyr Tyr Phe Asp
        275                 280                 285

Phe Asn Trp Val Thr Arg Ile Phe Leu Phe Leu His Ser Val Val Phe
    290                 295                 300

Val Met Lys Ser His Ser Phe Ala Phe Tyr Asn Gly Tyr Leu Trp Asp
305                 310                 315                 320

Ile Lys Gln Glu Leu Glu Tyr Ser Ser Lys Gln Leu Gln Lys Tyr Lys
                325                 330                 335

Glu Ser Leu Ser Pro Glu Thr Arg Glu Ile Leu Gln Lys Ser Cys Asp
            340                 345                 350

Phe Cys Leu Phe Glu Leu Asn Tyr Gln Thr Lys Asp Asn Asp Phe Pro
        355                 360                 365

Asn Asn Ile Ser Cys Ser Asn Phe Phe Met Phe Cys Leu Phe Pro Val
    370                 375                 380

Leu Val Tyr Gln Ile Asn Tyr Pro Arg Thr Ser Arg Ile Arg Trp Arg
385                 390                 395                 400

Tyr Val Leu Glu Lys Val Cys Ala Ile Ile Gly Thr Ile Phe Leu Met
                405                 410                 415

Met Val Thr Ala Gln Phe Phe Met His Pro Val Ala Met Arg Cys Ile
            420                 425                 430

Gln Phe His Asn Thr Pro Thr Phe Gly Gly Trp Ile Pro Ala Thr Gln
        435                 440                 445

Glu Trp Phe His Leu Leu Phe Asp Met Ile Pro Gly Phe Thr Val Leu
    450                 455                 460
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Tyr Met Leu Thr Phe Tyr Met Ile Trp Asp Ala Leu Leu Asn Cys Val
465 470 475 480

Ala Glu Leu Thr Arg Phe Ala Asp Arg Tyr Phe Tyr Gly Asp Trp Trp
485 490 495

Asn Cys Val Ser Phe Glu Glu Phe Ser Arg Ile Trp Asn Val Pro Val
500 505 510

His Lys Phe Leu Leu Arg His Val Tyr His Ser Ser Met Gly Ala Leu
515 520 525

His Leu Ser Lys Ser Gln Ala Thr Leu Phe Thr Phe Phe Leu Ser Ala
530 535 540

Val Phe His Glu Met Ala Met Phe Ala Ile Phe Arg Arg Val Arg Gly
545 550 555 560

Tyr Leu Phe Met Phe Gln Leu Ser Gln Phe Val Trp Thr Ala Leu Ser
565 570 575

Asn Thr Lys Phe Leu Arg Ala Arg Pro Gln Leu Ser Asn Val Val Phe
580 585 590

Ser Phe Gly Val Cys Ser Gly Pro Ser Ile Ile Met Thr Leu Tyr Leu
595 600 605

Thr Leu
610

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2421 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TATAAAATTC CTTTCATCAA TACATCTATA TATTCGAATA TATAGATAAA CCAATACAAA 60

AACATACTGA AATTTTTTGA AAACAACTAA AACTATTCAT TGCAGTTACA CGTGAATGCT 120

AAACTTTATA TCGCTCTTGT CGGTCCCGCG GAGTTAACAT TTAACGGCTT CTCGCGCAAT 180

AACCGGAAAA ATTCCAACAG TTTCTTTGTA ATATTATTAA GCCTTCTTTT TTCCCGGAAT 240

CTATAAGAGG GGACGAAAAT TAGCCGCTAT TAATTCTGGT ATTGCCACCT AGACAAGAAG 300

TAAACAGACA CATTACGTTA GCAAAAGCAA CAATAACAAA CACAACCATG GACAAGAAGA 360

AGGATCTACT GGAGAACGAA CAATTTCTCC GCATCCAAAA GCTCAACGCT GCCGATGCGG 420

GCAAAAGACA ATCTATAACA GTGGACGACG AGGGCGAACT ATATGGGTTA GACACCTCCG 480

GCAACTCACC AGCCAATGAA CACACAGCTA CCACAATTAC ACAGAATCAC AGCGTGGTGG 540

CCTCAAACGG AGACGTCGCA TTCATCCCAG GAACTGCTAC CGAAGGCAAT ACAGAGATTG 600

TAACTGAAGA AGTGATTGAG ACCGATGATA ACATGTTCAA GACCCATGTG AAGACTTTAA 660

GCTCCAAAGA GAAGGCACGG TATAGGCAAG GGTCCTCTAA CTTTATATCG TATTTCGATG 720

ATATGTCATT TGAACACAGG CCCAGTATAT TAGATGGGTC AGTTAACGAG CCCTTCAAGA 780

CCAAATTCGT GGGACCTACT TTAGAAAAGG AGATCAGAAG AGGGGAGAAA GAGCTAATGG 840

CCATGCGCAA AAATTTACAC CACCGCAAGT CCTCCCCAGA TGCTGTCGAC TCAGTAGGGA 900

AAAATGATGG CGCCGCCCCA ACTACTGTTC CAACTGCCGC CACCTCAGAA ACGGTGGTCA 960

CCGTTGAAAC CACCATAATT TCATCCAATT TCTCCGGGTT GTACGTGGCG TTTTGGATGG 1020

CTATTGCATT TGGTGCTGTC AAGGCTTTAA TAGACTATTA TTACCAGCAT AATGGTAGCT 1080

TCAAGGATTC GGAGATCTTG AAATTTATGA CTACGAATTT GTTCACTGTG GCATCCGTAG 1140

ATCTTTTGAT GTATTTGAGC ACTTATTTTG TCGTTGGAAT ACAATACTTA TGCAAGTGGG 1200

GGGTCTTGAA ATGGGGCACT ACCGGCTGGA TCTTCACCTC AATTTACGAG TTTTTGTTTG 1260

TTATCTTCTA CATGTATTTA ACAGAAAACA TCCTAAAACT ACACTGGCTG TCCAAGATCT 1320

TCCTTTTTTT GCATTCTTTA GTTTTATTGA TGAAAATGCA TTCTTTCGCC TTCTACAATG 1380

GCTATCTATG GGGTATAAAG GAAGAACTAC AATTTTCCAA AAGCGCTCTT GCCAAATACA 1440

AGGATTCTAT AAATGATCCA AAAGTTATTG GTGCTCTTGA GAAAAGCTGT GAGTTTTGTA 1500

GTTTTGAATT GAGCTCTCAG TCTTTAAGCG ACCAAACTCA AAAATTCCCC AACAATATCA 1560

GTGCAAAAAG CTTTTTTTGG TTCACCATGT TTCCAACCCT AATTTACCAA ATTGAATATC 1620

CAAGAACTAA GGAAATCAGA TGGAGCTACG TATTAGAAAA GATCTGCGCC ATCTTCGGTA 1680

CCATTTTCTT AATGATGATA GATGCTCAAA TCTTGATGTA TCCTGTAGCA ATGAGAGCAT 1740

TGGCTGTGCG CAATTCTGAA TGGACTGGTA TATTGGATAG ATTATTGAAA TGGGTTGGAT 1800

TGCTCGTTGA TATCGTCCCA GGGTTTATCG TGATGTACAT CTTGGACTTC TATTTGATTT 1860

GGGATGCCAT TTTGAACTGT GTGGCTGAAT TGACAAGATT TGGCGACAGA TATTTCTACG 1920

GTGACTGGTG GAATTGTGTT AGTTGGGCAG ACTTCAGTAG AATTTGGAAC ATCCCAGTGC 1980

ATAAGTTTTT GTTAAGACAT GTTTACCATA GTTCAATGAG TTCATTCAAA TTGAACAAGA 2040

GTCAAGCAAC TTTGATGACC TTTTTCTTAA GTTCCGTCGT TCATGAATTA GCAATGTACG 2100

TTATCTTCAA GAAATTGAGG TTTTACTTGT TCTTCTTCCA AATGCTGCAA ATGCCATTAG 2160

TAGCTTTAAC AAATACTAAA TTCATGAGGA ACAGAACCAT AATCGGAAAT GTTATTTTCT 2220

GGCTCGGTAT CTGCATGGGA CCAAGTGTCA TGTGTACGTT GTACTTGACA TTCTAAGGCA 2280

TCCTGCAACT GTTCTGTGGA GCTATTAAAT CTTTATAGTA AATTTTTTTT TACTTTTTTT 2340

TTTTTTTTTT TTTTTTTTTA TTATTTACAA GCGTCTATAT ATTTTCTATT ATAGAATATT 2400

GTCATTTATT ACATTGGTTC A

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 642 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Amino Acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Asp Lys Lys Lys Asp Leu Leu Glu Asn Glu Gln Phe Leu Arg Ile
1               5                  10                  15

Gln Lys Leu Asn Ala Ala Asp Ala Gly Lys Arg Gln Ser Ile Thr Val
            20                  25                  30

Asp Asp Glu Gly Glu Leu Tyr Gly Leu Asp Thr Ser Gly Asn Ser Pro
        35                  40                  45

Ala Asn Glu His Thr Ala Thr Thr Ile Thr Gln Asn His Ser Val Val
    50                  55                  60

Ala Ser Asn Gly Asp Val Ala Phe Ile Pro Gly Thr Ala Thr Glu Gly
65                  70                  75                  80

Asn Thr Glu Ile Val Thr Glu Glu Val Ile Glu Thr Asp Asp Asn Met
                85                  90                  95

Phe Lys Thr His Val Lys Thr Leu Ser Ser Lys Glu Lys Ala Arg Tyr
            100                 105                 110

Arg Gln Gly Ser Ser Asn Phe Ile Ser Tyr Phe Asp Asp Met Ser Phe
        115                 120                 125

Glu His Arg Pro Ser Ile Leu Asp Gly Ser Val Asn Glu Pro Phe Lys
    130                 135                 140

Thr Lys Phe Val Gly Pro Thr Leu Glu Lys Glu Ile Arg Arg Arg Glu
145                 150                 155                 160

Lys Glu Leu Met Ala Met Arg Lys Asn Leu His His Arg Lys Ser Ser
                165                 170                 175

Pro Asp Ala Val Asp Ser Val Gly Lys Asn Asp Gly Ala Ala Pro Thr
            180                 185                 190

Thr Val Pro Thr Ala Ala Thr Ser Glu Thr Val Val Thr Val Glu Thr
        195                 200                 205

Thr Ile Ile Ser Ser Asn Phe Ser Gly Leu Tyr Val Ala Phe Trp Met
    210                 215                 220

Ala Ile Ala Phe Gly Ala Val Lys Ala Leu Ile Asp Tyr Tyr Tyr Gln
225                 230                 235                 240

His Asn Gly Ser Phe Lys Asp Ser Glu Ile Leu Lys Phe Met Thr Thr
                245                 250                 255
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Asn Leu Phe Thr Val Ala Ser Val Asp Leu Leu Met Tyr Leu Ser Thr
            260                 265                 270

Tyr Phe Val Val Gly Ile Gln Tyr Leu Cys Lys Trp Gly Val Leu Lys
        275                 280                 285

Trp Gly Thr Thr Gly Trp Ile Phe Thr Ser Ile Tyr Glu Phe Leu Phe
    290                 295                 300

Val Ile Phe Tyr Met Tyr Leu Thr Glu Asn Ile Leu Lys Leu His Trp
305                 310                 315                 320

Leu Ser Lys Ile Phe Leu Phe Leu His Ser Leu Val Leu Leu Met Lys
                325                 330                 335

Met His Ser Phe Ala Phe Tyr Asn Gly Tyr Leu Trp Gly Ile Lys Glu
            340                 345                 350

Glu Leu Gln Phe Ser Lys Ser Ala Leu Ala Lys Tyr Lys Asp Ser Ile
        355                 360                 365

Asn Asp Pro Lys Val Ile Gly Ala Leu Glu Lys Ser Cys Glu Phe Cys
    370                 375                 380

Ser Phe Glu Leu Ser Ser Gln Ser Leu Ser Asp Gln Thr Gln Lys Phe
385                 390                 395                 400

Pro Asn Asn Ile Ser Ala Lys Ser Phe Phe Trp Phe Thr Met Phe Pro
                405                 410                 415

Thr Leu Ile Tyr Gln Ile Glu Tyr Pro Arg Thr Lys Glu Ile Arg Trp
            420                 425                 430

Ser Tyr Val Leu Glu Lys Ile Cys Ala Ile Phe Gly Thr Ile Phe Leu
        435                 440                 445

Met Met Ile Asp Ala Gln Ile Leu Met Tyr Pro Val Ala Met Arg Ala
    450                 455                 460

Leu Ala Val Arg Asn Ser Glu Trp Thr Gly Ile Leu Asp Arg Leu Leu
465                 470                 475                 480

Lys Trp Val Gly Leu Leu Val Asp Ile Val Pro Gly Phe Ile Val Met
                485                 490                 495

Tyr Ile Leu Asp Phe Tyr Leu Ile Trp Asp Ala Ile Leu Asn Cys Val
            500                 505                 510

Ala Glu Leu Thr Arg Phe Gly Asp Arg Tyr Phe Tyr Gly Asp Trp Trp
        515                 520                 525

Asn Cys Val Ser Trp Ala Asp Phe Ser Arg Ile Trp Asn Ile Pro Val
    530                 535                 540

His Lys Phe Leu Leu Arg His Val Tyr His Ser Ser Met Ser Ser Phe
545                 550                 555                 560

Lys Leu Asn Lys Ser Gln Ala Thr Leu Met Thr Phe Phe Leu Ser Ser
                565                 570                 575

Val Val His Glu Leu Ala Met Tyr Val Ile Phe Lys Lys Leu Arg Phe
            580                 585                 590
```

Tyr Leu Phe Phe Phe Gln Met Leu Gln Met Pro Leu Val Ala Leu Thr
595 600 605

Asn Thr Lys Phe Met Arg Asn Arg Thr Ile Ile Gly Asn Val Ile Phe
610 615 620

Trp Leu Gly Ile Cys Met Gly Pro Ser Val Met Cys Thr Leu Tyr Leu
625 630 635 640

Thr Phe

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 983 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATGGAGCTCA ACTTTCCCCG CTCTCCCCGC ATCCGGAAGC GCTTTCTGCT GCGACGGATC 60

CTTGAGATGC TGTTCTTCAC CCAGCTCCAG GTGGGGCTGA TCCAGCAGTG GATGGTCCCC 120

ACCATCCAGA ACTCCATGAA GCCCTTCAAG GACATGGACT ACTCACGCAT CATCGAGCGC 180

CTCCTGAAGC TGGCGGTCCC CAATCACCTC ATCTGGCTCA TCTTCTTCTA CTGGCTCTTC 240

CACTCCTGCC TGAATGCCGT GGCTGAGCTC ATGCAGTTTG GAGACCGGGA GTTCTACCGG 300

GACTGGTGGA ACTCCGAGTC TGTCACCTAC TTCTGGCAGA ACTGGAACAT CCCTGTGCAC 360

AAGTGGTGCA TCAGACACTT CTACAAGCCC ATGCTTCGAC GGGGCAGCAG CAAGTGGATG 420

GCCAGGACAG GGGTGTTCCT GGCCTCGGCT TTCTTCCACG AGTACCTGGT GAGCGTCCCT 480

CTGCGAATGT TCCGCCTCTG GGCTTTCACG GGCATGATGG CTCAGATCCC ACTGGCCTGG 540

TTCGTGGGCC GCTTTTTCCA GGGCAACTAT GGCAACGCAG CTGTGTGGCT GTCGCTCATC 600

ATCGGACAGC CAATAGCCGT CCTCATGTAC GTCCACGAAC TACTACGTGC TCAACTATGA 660

GGCCCCAGCG GCAGAGGCCT GAGCTGCACC TGAGGGCCTG GCTTCTCACT GCCACCTCAA 720

ACCCGCTGCC AGAGCCCACC TCTCCTCCTA GGCCTCGAGT GCTGGGGATG GGCCTGGCTG 780

CACAGCATCC TCCTCTGGTC CCAGGGAGGC CTCTCTGCCC TATGGGGCTC TGTCCTGCAC 840

CCCTCAGGGA TGGCGACAGC AGGCCAGACA CAGTCTGATG CCAGCTGGGA GTCTTGCTGA 900

CCCTGCCCCG GGTCCGAGGG TGTCAATAAA GTGCTGTCCA GTGAGAAAAA GAAAAAAAAA 960

AAAAAAAAAA ATTCTGCGGC CGC

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 219 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Amino Acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Met Glu Leu Asn Phe Pro Arg Ser Pro Arg Ile Arg Lys Arg Phe Leu
1               5                  10                  15

Leu Arg Arg Ile Leu Glu Met Leu Phe Phe Thr Gln Leu Gln Val Gly
            20                  25                  30

Leu Ile Gln Gln Trp Met Val Pro Thr Ile Gln Asn Ser Met Lys Pro
        35                  40                  45

Phe Lys Asp Met Asp Tyr Ser Arg Ile Ile Glu Arg Leu Leu Lys Leu
    50                  55                  60

Ala Val Pro Asn His Leu Ile Trp Leu Ile Phe Phe Tyr Trp Leu Phe
65                  70                  75                  80

His Ser Cys Leu Asn Ala Val Ala Glu Leu Met Gln Phe Gly Asp Arg
                85                  90                  95

Glu Phe Tyr Arg Asp Trp Trp Asn Ser Glu Ser Val Thr Tyr Phe Trp
            100                 105                 110

Gln Asn Trp Asn Ile Pro Val His Lys Trp Cys Ile Arg His Phe Tyr
        115                 120                 125

Lys Pro Met Leu Arg Arg Gly Ser Ser Lys Trp Met Ala Arg Thr Gly
    130                 135                 140

Val Phe Leu Ala Ser Ala Phe Phe His Glu Tyr Leu Val Ser Val Pro
145                 150                 155                 160

Leu Arg Met Phe Arg Leu Trp Ala Phe Thr Gly Met Met Ala Gln Ile
                165                 170                 175

Pro Leu Ala Trp Phe Val Gly Arg Phe Phe Gln Gly Asn Tyr Gly Asn
            180                 185                 190

Ala Ala Val Trp Leu Ser Leu Ile Ile Gly Gln Pro Ile Ala Val Leu
        195                 200                 205

Met Tyr Val His Glu Leu Leu Arg Ala Gln Leu
    210                 215
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 455 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ATGTTGAACT TCATGATGCA TGACCAGCGC ACCGGCCCGG CATGGAACGT GCTGATGTGG 60

ACCATGCTGT TTCTAGGCCA GGGAATCCAG GTCAGCCTGT ACTGCCAGGA GTGGTACGCA 120

CGGACGCACT GCCCCTTACC CCAGGCAACT TTCTGGGGGC TGGTGACACC TCGATCTTGG 180

TCCTGCCATA CCTAGAGGTC GGGACAGACG ACGCTACCTG CCCAGACACC ACCAAGTTCT 240

CTGCCTGCAA AACCTGGGGA CCAGGACTTC CTGTCTTGCA TTCCCAAATT TGGGTTCTTG 300

AGTCGAGGCA ACCTTGCACA CAAGACCCCA CCAAGGGATT GTTGCAAGGG ATTAGATTTT 360

GCAGATTTGT TGGGTAATGA TTCAACGACT CAGCTGGGGG TTGACCAGGG TTGATTTTTC 420

AATCCTTTTC CCCTGGGTTT GGGTTACAGG TTTTT 455

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 64 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Amino Acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Leu Asn Phe Met Met His Asp Gln Arg Thr Gly Pro Ala Trp Asn
1 5 10 15

Val Leu Met Trp Thr Met Leu Phe Leu Gly Gln Gly Ile Gln Val Ser
20 25 30

Leu Tyr Cys Gln Glu Trp Tyr Ala Arg Thr His Cys Pro Leu Pro Gln
35 40 45

Ala Thr Phe Trp Gly Leu Val Thr Pro Arg Ser Trp Ser Cys His Thr
50 55 60

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 517 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ATGGACAACG CGGGGTCTGA TACGACTCAC TATAGGGAAT TTGGCCCTCG AGCAGTAGAT 60

TCGGCACGAT GGGCACGAGG ACTCCATCAT GTTCCTCAAG CTTTATTCCT ACCGGGATGT 120

CAACCTGTGG TGCCGCCAGC GAAGGGTCAA GGCCAAAGCT GTCTCTACAG GGAAGAAGGT 180

CAGTGGGGCT GCTGCGAGCA AGCTGTGAGC TATCCAGACA ACCTGACCTA CCGAGATCTC 240

GATTACTTCA TCTTTGCTCC TACTTTGTGT TATGAACTCA ACTTTCCTCG GTCCCCCCGA 300

ATACGAGAGC GCTTTCTGCT ACGACGAGTT CTTGAGATGC TCTTTTTTAC CCAGCTTCAA 360

GTGGGGCTGA TCCAACAGTG GATGGTCCCT ACTATCCAGA ACTCCATGGA AGCCCTTTCA 420

AGAGCTTCTG GCAGTTTTGG AGACCGCGAG TTCTACAGAG ATTGGTGGAA TGCTGAGTCT 480

GTCACCGACT TTTGGCAGAA CTGGAATATC CCCGTGG 517

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 172 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Amino Acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met Asp Asn Ala Gly Ser Asp Thr Thr His Tyr Arg Glu Phe Gly Pro
1 5 10 15

Arg Ala Val Asp Ser Ala Arg Trp Ala Arg Gly Leu His His Val Pro
20 25 30

Gln Ala Leu Phe Leu Pro Gly Cys Gln Pro Val Val Pro Pro Ala Lys
35 40 45

Gly Gln Gly Gln Ser Cys Leu Tyr Arg Glu Glu Gly Gln Trp Gly Cys
50 55 60

Cys Glu Gln Ala Val Ser Tyr Pro Asp Asn Leu Thr Tyr Arg Asp Leu
65 70 75 80

Asp Tyr Phe Ile Phe Ala Pro Thr Leu Cys Tyr Glu Leu Asn Phe Pro
85 90 95

Arg Ser Pro Arg Ile Arg Glu Arg Phe Leu Leu Arg Arg Val Leu Glu
100 105 110

Met Leu Phe Phe Thr Gln Leu Gln Val Gly Leu Ile Gln Gln Trp Met
115 120 125

Val Pro Thr Ile Gln Asn Ser Met Glu Ala Leu Ser Arg Ala Ser Gly
130 135 140

Ser Phe Gly Asp Arg Glu Phe Tyr Arg Asp Trp Trp Asn Ala Glu Ser

145 150 155 160

Val Thr Asp Phe Trp Gln Asn Trp Asn Ile Pro Val
165 170

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 366 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Amino Acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Met Lys Asp Leu Leu Glu Phe Leu Lys Ile Arg Leu Asn Ala Asp Ala
1 5 10 15

Lys Arg Ser Thr Asp Ser Pro Thr Val Ser Glu Val Glu Arg Gly Lys
20 25 30

Gln Glu Ile Glu Ala His Lys Ser Lys Lys Arg Phe Arg Ser Phe Ser
35 40 45

Phe Phe Asp Ser Phe Glu Arg Pro Ser Leu Leu Asp Gly Asn Pro Phe
50 55 60

Thr Thr Phe Gly Pro Val Leu Glu Lys Glu Lys Asn Leu His Lys Lys
65 70 75 80

Lys Thr Thr Val Thr Asp Val Ser Asn Phe Ser Gly Ile Tyr Val Phe
85 90 95

Trp Met Leu Ala Leu Asp Tyr Tyr Gly Glu Ile Leu Tyr Met Thr Thr
100 105 110

Leu Phe Thr Val Ala Asp Leu Met Phe Leu Ser Thr Phe Phe Val Val
115 120 125

Leu Lys Trp Thr Gly Ile Ser Ile Glu Phe Leu Phe Ile Phe Leu Trp
130 135 140

Ser Arg Ile Phe Leu Phe Leu His Ser Val Phe Val Met Lys His Ser
145 150 155 160

Phe Ala Phe Tyr Asn Gly Tyr Leu Trp Ile Lys Glu Glu Leu Ser Leu
165 170 175

Lys Tyr Lys Glu Ser Ser Pro Leu Gln Lys Ser Cys Phe Cys Phe Glu
180 185 190

Leu Gln Phe Pro Asn Asn Ile Ser Phe Phe Phe Phe Pro Thr Leu Ile
195 200 205

Tyr Gln Ile Tyr Pro Arg Thr Ile Arg Trp Tyr Val Leu Glu Lys Cys
210 215 220

Ala Ile Phe Gly Thr Ile Phe Leu Met Met Ala Gln Met Pro Val Ala

```
225                 230                 235                 240

Met Arg Asn Phe Trp Gln Leu Leu Asp Ile Pro Gly Phe Val Leu Tyr
                245                 250                 255

Leu Thr Phe Tyr Ile Trp Asp Ala Leu Asn Cys Val Ala Glu Leu Thr
            260                 265                 270

Arg Phe Gly Asp Arg Tyr Phe Tyr Gly Asp Trp Trp Asn Cys Val Ser
        275                 280                 285

Phe Ser Arg Ile Trp Asn Val Pro Val His Lys Phe Leu Leu Arg His
    290                 295                 300

Val Tyr His Ser Ser Met Phe Lys Leu Lys Ser Gln Ala Thr Leu Thr
305                 310                 315                 320

Phe Phe Leu Ser Ala Val Val His Glu Ala Met Val Ile Phe Arg Tyr
                325                 330                 335

Leu Phe Phe Gln Gln Met Ala Leu Asn Thr Lys Phe Arg Arg Ile Asn
            340                 345                 350

Val Phe Trp Gly Cys Gly Pro Ser Val Thr Leu Tyr Leu Thr
        355                 360                 365
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 96 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Amino Acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Pro Asn His Leu Ile Trp Leu Ile Phe Phe Tyr Trp Leu Phe His Ser
1               5                   10                  15

Cys Leu Asn Ala Val Ala Glu Leu Met Gln Phe Gly Asp Arg Glu Phe
            20                  25                  30

Tyr Arg Asp Trp Trp Asn Ser Glu Ser Val Thr Tyr Phe Trp Gln Asn
        35                  40                  45

Trp Lys Ile Pro Val His Lys Trp Cys Ile Arg His Phe Tyr Lys Pro
    50                  55                  60

Met Leu Arg Arg Gly Ser Ser Lys Trp Met Ala Arg Asp Arg Gly Val
65                  70                  75                  80

Pro Gly Pro Ser Ala Phe Phe His Val Val Thr Trp Val Ser Val Pro
                85                  90                  95
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 91 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GAGGGGACGA AAATTAGCCG CTATTAATTC TGGTATTGCC ACCTAGACAA GAAGTAAACA 60

GACACAGATG CAAGAGTTCG AATCTCTTAG C 91

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 76 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CTATAAAGAT TTAATAGCTC CACAGAACAG TTGCAGGATG CCTTAGGGTC GACTACGTCG 60

TAAGGCCGTT TCTGAC 76

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CATTGCAGTT ACACGTGAAT GC 22

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

TAGCTCCACA GAACAGTTGC AGG 23

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CTCTGACAAC AACGAAGTCA G 21

What is claimed is:

1. An isolated nucleic acid which encodes an acylcoenzyme A: cholesterol acyltransferase II.

2. An isolated nucleic acid which encodes an acylcoenzyme A: cholesterol acyltransferase III.

3. The isolated nucleic acid of claim 1 or 2, wherein the nucleic acid is DNA or RNA.

4. The isolated nucleic acid of claim 3, wherein the nucleic acid is cDNA or genomic DNA.

5. The isolated nucleic acid of claim 1 comprising a nucleic acid having the sequence as set forth in Figure 15.

6. The isolated nucleic acid of claim 1, wherein the nucleic acid encodes a human wildtype acylcoenzyme A: cholesterol acyltransferase II having substantially the same amino acid sequence as set forth in Figure 15.

7. The isolated nucleic acid of claim 2, comprising a nucleic acid having the sequence as set forth in Figure 16.

8. The isolated nucleic acid of claim 2, wherein the nucleic acid encodes a human wildtype acylcoenzyme A: cholesterol acyltransferase III having substantially the same amino acid sequence as set forth in Figure 16.

9. The isolated nucleic acid of claim 1 comprising a

nucleic acid having the sequence designated Seq. I.D. No.: 11.

10. The isolated nucleic acid of claim 1, wherein the nucleic acid encodes a mouse wildtype acylcoenzyme A: cholesterol acyltransferase II having substantially the same amino acid sequence as the sequence designated Seq. I.D. No.: 12.

11. The isolated nucleic acid of claim 1, wherein the nucleic acid encodes a mutant acylcoenzyme A: cholesterol acyltransferase II.

12. The isolated nucleic acid of claim 2, wherein the nucleic acid encodes a mutant acylcoenzyme A: cholesterol acyltransferase III.

13. A vector comprising the isolated nucleic acid of claim 1 or 2.

14. The vector of claim 13 further comprising a promoter of RNA transcription operatively linked to the nucleic acid.

15. The vector of claim 14, wherein the promoter comprises a bacterial, yeast, insect or mammalian promoter.

16. The vector of claim 14, further comprising plasmid, cosmid, yeast artificial chromosome (YAC), bacteriophage or eukaryotic viral DNA.

17. The vector of claim 14 designated YEpAB-ACAT2.

18. A host vector system for the production of a

polypeptide which comprises the vector of claim 14 in a suitable host.

19. The host vector system of claim 18, wherein the suitable host is a prokaryotic or eukaryotic cell.

20. The host vector system of claim 19, wherein the prokaryotic cell is a bacterial cell.

21. The host vector system of claim 19, wherein the eukaryotic cell is a yeast, insect, plant or mammalian cell.

22. A method for producing a polypeptide which comprises growing the host vector system of claim 18 under suitable conditions permitting production of the polypeptide and recovering the polypeptide so produced.

23. A method of obtaining a polypeptide in purified form which comprises:

(a) introducing the vector of claim 14 into a suitable host cell;

(b) culturing the resulting cell so as to produce the polypeptide;

© recovering the polypeptide produced in step (b); and

(d) purifying the polypeptide so recovered.

24. A purified wildtype acylcoenzyme A: cholesterol acyltransferase II.

25. A purified mutant acylcoenzyme A: cholesterol acyltransferase II.

26. A purified wildtype acylcoenzyme A: cholesterol acyltransferase III.

27. A purified mutant acylcoenzyme A: cholesterol acyltransferase III.

28. An oligonucleotide of at least 15 nucleotides capable of specifically hybridizing with a unique sequence of nucleotides present within a nucleic acid which encodes a wildtype acylcoenzyme A: cholesterol acyltransferase II without hybridizing to a nucleic acid which encodes a mutant acylcoenzyme A: cholesterol acyltransferase II.

29. An oligonucleotide of at least 15 nucleotides capable of specifically hybridizing with a unique sequence of nucleotides present within the nucleic acid which encodes a mutant acylcoenzyme A: cholesterol acyltransferase II without hybridizing to a nucleic acid which encodes a wildtype acylcoenzyme A: cholesterol acyltransferase II.

30. An oligonucleotide of at least 15 nucleotides capable of specifically hybridizing with a unique sequence of nucleotides present within a nucleic acid which encodes a wildtype acylcoenzyme A: cholesterol acyltransferase III without hybridizing to a nucleic acid which encodes a mutant acylcoenzyme A: cholesterol acyltransferase III.

31. An oligonucleotide of at least 15 nucleotides capable of specifically hybridizing with a unique sequence of nucleotides present within the nucleic acid which encodes a mutant acylcoenzyme A: cholesterol acyltransferase III without hybridizing

to a nucleic acid which encodes a wildtype acylcoenzyme A: cholesterol acyltransferase III.

32. The oligonucleotide of claim 28, 29, 30 or 31 wherein the nucleic acid is DNA or RNA.

33. A nucleic acid having a sequence complementary to the sequence of the isolated nucleic acid of claim 1 or 2.

34. A method for determining whether a subject known to have an imbalance in sterol levels has the imbalance due to a defect in esterification of sterol which comprises:

(a) obtaining from the subject an appropriate sample containing a mixture of all of the subject's nucleic acids; and

(b) determining whether any nucleic acid in the sample from step (a) is, or is derived from, a nucleic acid which encodes a mutant acylcoenzyme A: cholesterol acyltransferase so as to thereby determine whether the subject's imbalance in sterol levels is due to a defect in esterification of sterol.

35. The method of claim 34, wherein the determining of step (b) comprises:

(I) contacting the sample of step (a) with the isolated nucleic acid of claim 11 or 12 or the oligonucleotide of claim 29 or 31 under conditions permitting binding of any nucleic acid in the sample which is, or is derived from, a nucleic acid which encodes a mutant acylcoenzyme A: cholesterol acyltransferase to the nucleic acid or

oligonucleotide so as to form a complex;

(ii) isolating the complex so formed; and

(iii) identifying the nucleic acid in the isolated complex so as to thereby determine whether any nucleic acid in the sample contains a nucleic acid which is, or is derived from, a nucleic acid which encodes a mutant acylcoenzyme A: cholesterol acyltransferase II or III.

36. The method of claim 35, wherein the isolated nucleic acid or the oligonucleotide is labeled with a detectable marker.

37. The method of claim 36, wherein the detectable marker is a radioactive isotope, a fluorophore or an enzyme.

38. The method of claim 35, wherein the nucleic acid sample is first bound to a solid matrix before performing step (I).

39. The method of claim 35, wherein the sample comprises blood or sera.

40. A method for treating a subject who has an imbalance in sterol levels due to a defect in esterification of sterol which comprises introducing the isolated nucleic acid of claim 1 or 2 into the subject under conditions such that the nucleic acid expresses a wildtype acylcoenzyme A: cholesterol acyltransferase II or III, so as to thereby treat the subject.

41. A method for inhibiting wildtype acylcoenzyme A: cholesterol acyltransferase II or III in a subject

which comprises transforming appropriate cells from the subject with a vector which expresses the nucleic acid of claim 33, and introducing the transformed cells into the subject so as to thereby inhibit wildtype acylcoenzyme A: cholesterol acyltransferase II or III.

42. The method of claim 41, wherein the nucleic acid of claim 33 is capable of specifically hybridizing to a mRNA molecule encoding acylcoenzyme A: cholesterol acyltransferase II or III so as to prevent translation of the mRNA molecule.

43. A method for inhibiting the wildtype acylcoenzyme A: cholesterol acyltransferase II or III in a subject which comprises introducing the oligonucleotide of claim 28 or 30 into the subject so as to thereby inhibit the wildtype acylcoenzyme A: cholesterol acyltransferase II or III.

44. The method of claim 43, wherein the oligonucleotide of clam 28 or 30 is capable of specifically hybridizing to a mRNA molecule encoding acylcoenzyme A: cholesterol acyltransferase II or III so as to prevent translation of the mRNA molecule.

45. A method for identifying a chemical compound which is capable of inhibiting acylcoenzyme A: cholesterol acyltransferase II or III in a subject which comprises:

(a) contacting a wildtype acylcoenzyme A: cholesterol acyltransferase II or III with the chemical compound under conditions permitting binding between the acylcoenzyme and the chemical compound;

(b) detecting specific binding of the chemical compound to the acylcoenzyme; and

© determining whether the chemical compound inhibits the activity of the coenzyme so as to identify a chemical compound which is capable of inhibiting acylcoenzyme A: cholesterol acyltransferase II ir III in a subject.

46. A pharmaceutical composition comprising the chemical compound identified by the method of claim 45 in an amount effective to inhibit acylcoenzyme A: cholesterol acyltransferase II or III in a subject and a pharmaceutically effective carrier.

47. A method of treating a subject who has atherosclerosis comprising administering the pharmaceutical composition of claim 46 to the subject.

48. A method of treating a subject who has hyperlipidemia comprising administering the pharmaceutical composition of claim 46 to the subject.

49. A transgenic, nonhuman mammal comprising the isolated nucleic acid of claim 1 or 2.

FIG. 1A

```
          1
ARE1      MTETKDLLQD EEFLKIRRLN SAEANKRHSV TYDN..VILP QESMEVSPRS STTSLV.... .EPVESTEGV ESTEAERVAG KQEQEEEYPV DAHMQKYLSH
ARE2      MDKKKDLLEN EQFLRIQKLN AADAGKRQSI TVDDEGELYG LDTSGNSPAN EHTATTITQN HSVVASNGDV AFIPGTATEG NTEIVTEEVI ETDDNMFKTH
hACAT     MVGEEKMSLR NRLSKSRENP EEDEDQRNP. .......... .......... .......... .....AKESL ETPSNGRIDI KQLIAKKIKL TAEAEEL...
CON       M---KDLL-- E-FLKIR-LN -ADA-KR-S- T-D------- ------SP-- --T------- ---V-S-E-V E-----R--G KQEI--E--- -A-------H

          101
ARE1      LK...SKSRS RFHRKDASKY VSFFGDVSFD PRPTLLDSAI NVPFQTTFKG PVLEKQL... ........KN LQLTK..... ....TKTKAT VKTTVKTTEK
ARE2      VKTLSSKEKA RY.RQGSSNF ISYFDDMSFE HRPSILDGSV NEPFKTKFVG PTLEKEIRRR EKELMAMRKN LHHRKSSPDA VDSVGKNDGA APTTVPTAAT
hACAT     ........KP FFMKEVGSHF DDFVTNL.IE KSASLDNGGC AL...TTF.. SVLEGE.... ........KN NHRAKDLRAP PE.......Q GKIFIARRSL
CON       -K---SK-K- RF-R---S-F -SFF-D-SFE -RPSLLDG-- N-PF-TTF-G PVLEKE---- --------KN LH--K----- -----K---- -KTTV-T---

          201
ARE1      TDKADAPPGE KLESNFSGIY VFAWMFLGWI AIRCCTDYYA SYGSAWNKLE IVQYMTTDLF TIAMLDLAMF LCTFFVVFVH WLVKKRIINW KWTGFVAVSI
ARE2      SETVVTVETT IISSNFSGLY VAFWMAIAFG AVKALIDYYY QHNGSFKDSE ILKFMTTNLF TVASVDLLMY LSTYFVVGIQ YLCKWGVLKW GTTGWIFTSI
hACAT     LDELLEVD.. ....HIRTIY HMFIALLILF ILSTLVVDYI DEGRLVLEFS LLSYAFGKFP TVVWTWWIMF LSTFSVPYFL FQHWRTGYSK SSHPLIRSLF
CON       -D----V--- ---SNFSGIY V-FWM-L--- A---L-DYY- --G------E IL-YMTT-LF TVA--DL-MF LSTFFVV--- -L-K-----W --TG-I--SI

          301
ARE1      FELAFIPVTF PIYVY..... ..YFDFNWVT RIFLFLHSVV FVMKSHSFAF YNGYLWDIKQ ELEYSSKQLQ KYKESLS.PE TREILQKSCD FCLFELNYQT
ARE2      YEFLFVIFYM YLTEN..... ..ILKLHWLS KIFLFLHSLV LLMKMHSFAF YNGYLWGIKE ELQFSKSALA KYKDSINDPK VIGALEKSCE FCSFELSSQS
hACAT     HGFLFMIFQI GVLGFGPTYV VLAYTLPPAS RFIIIFEQIR FVMKAHSF.. .......VRE NV...PRVLN SAKEKSSTVP IPTVNQ.... ..........
CON       -EFLF-IF-- ---------- -----L-W-S RIFLFLHS-V FVMK-HSFAF YNGYLW-IKE EL--S---L- KYKES-S-P- ----LQKSC- FC-FEL--Q-
```

FIG. 1B

```
      401
ARE1  KDND...FPN NISCSNFFMF CLFPVLVYQI NYPRTSRIRW RYVLEKVCAI IGTIFLMMVT AQFFMHPVAM RCIQFHNTPT FGGWIPATQE WFHLLFDMIP
ARE2  LSDQTQKFPN NISAKSFFWF TMFPTLIYQI EYPRTKEIRW SYVLEKICAI FGTIFLMMID AQILMYPVAM RALAVRN.SE WTGILDRLLK WVGLLVDIVP
hACAT ..........  ......YLYF LFAPTLIYRD SYPRNPTVRW GYVAMKFAQV FGCFFYVY.. ..YIFERLCA PLFRNIKQEP FSARVLV... .LCVFNSILP
CON   -------FPN NIS---FF-F --FPTLIYQI -YPRT--IRW -YVLEK-CAI FGTIFLMM-- AQ--M-PVAM R-----N--- F-G------- W--LL-DI-P
R07932                                                                                                          P
                                                                                                                .
      501
ARE1  GFTVLYMLTF YMIWDALLNC VAELTRFADR YFYGDWWNCV SFEEFSRIWN VPVHKFLLRH VYHSSMGAL. HLSKSQATLF .TFFLSAVFH EMAMFAIFRR
ARE2  GFIVMYILDF YLIWDAILNC VAELTRFGDR YFYGDWWNCV SWADFSRIWN IPVHKFLLRH VYHSSMSSF. KLNKSQATLM .TFFLSSVVH ELAMYVIFKK
hACAT GVLILF.LTF FAFLHCWLNA FAEMLRFGDR MFYKDWWNST SYSNYYRTWN VVVHDWLYYY AYKDFLWFFS KRFKSAAMLA .VFAVSAVVH EYALAVCLSF
CON   GF-VLY-LTF Y-IWDA-LNC VAELTRFGDR YFYGDWWNCV S---FSRIWN VPVHKFLLRH VYHSSM--F- KL-KSQATL- .TFFLSAVVH E-AM-VIF--
R07932 NHLIWLIF.F YWLFHSCLNA VAELMQFGDR EFYRDWWNSE SVTYFWQNWK IPVHKWCIRH FYKPMLRRGS ..SKWMARDR GVPGPSAFFH VVTW VSV P
         **  *  * *   *  *** **** ****  ** *****  *   *   *  ******  ** **   *   *   **  *     *   ** **       *

      601
ARE1  VRGYLFMFQL SQF VWTALS NTKFLRARPQ LSNVVFSFGV CSGPSIIMTL YLTL..... ..........  .......... ..
ARE2  LRFYLFFFQM LQ MPLVALT NTKFMRNRTI IGNVIFWLGI CMGPSVMCTL YLTFZ..... ..........  .......... ..
hACAT FYPVLFVLFM FFGMAFNFIV NDS..RKKP. IWNVLMWTSL FLGNGVLLCF YSQEWYARRHC PLKNPTFLDY VRPRSWTCRY VF
CON   -R-YLF-FQ- -Q-M---AL- NTKF-R-R-- I-NV-FW-G- C-GPSV--TL YLT-------- ---------- ---------- --
```

FIG. 2A

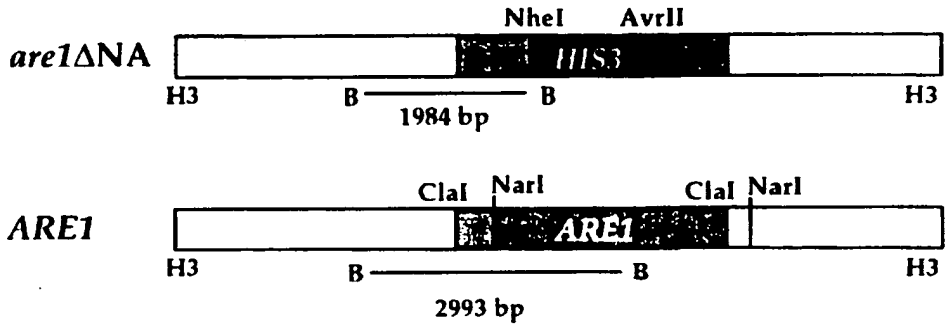

are1ΔNA
NheI
AvrII
HIS3
H3
B
B
H3
1984 bp
ARE1
ClaI
NarI
ClaI
NarI
ARE1
H3
B
B
H3
2993 bp

FIG. 2B

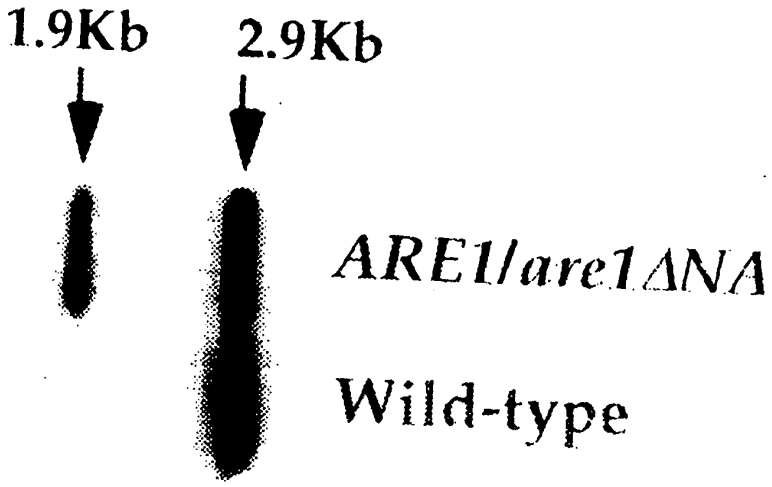


FIG. 2C

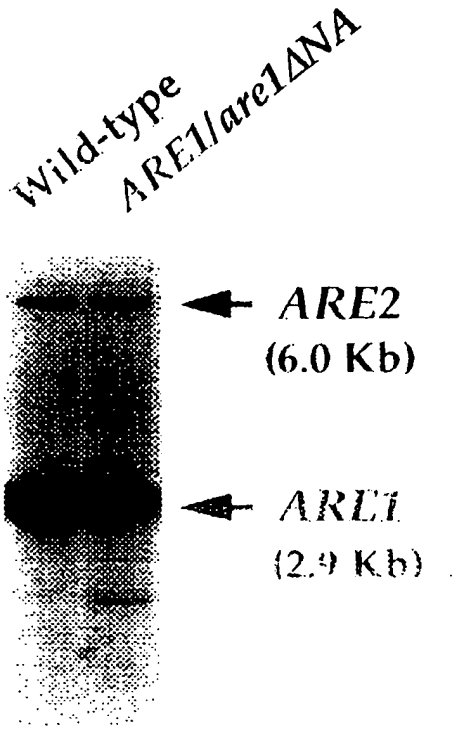


FIG. 2D

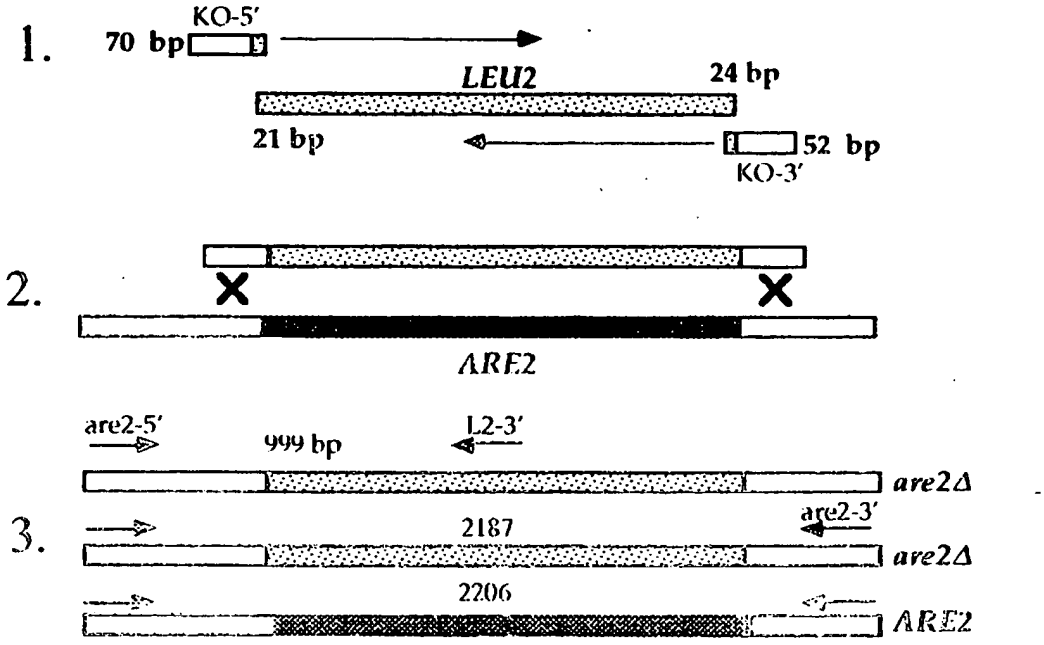

1.
KO-5'
70 bp
LEU2
24 bp
21 bp
52 bp
KO-3'
2.
ARE2
are2-5'
999 bp
L2-3'
are2Δ
3.
2187
are2-3'
are2Δ
2206
ARE2

FIG. 2E

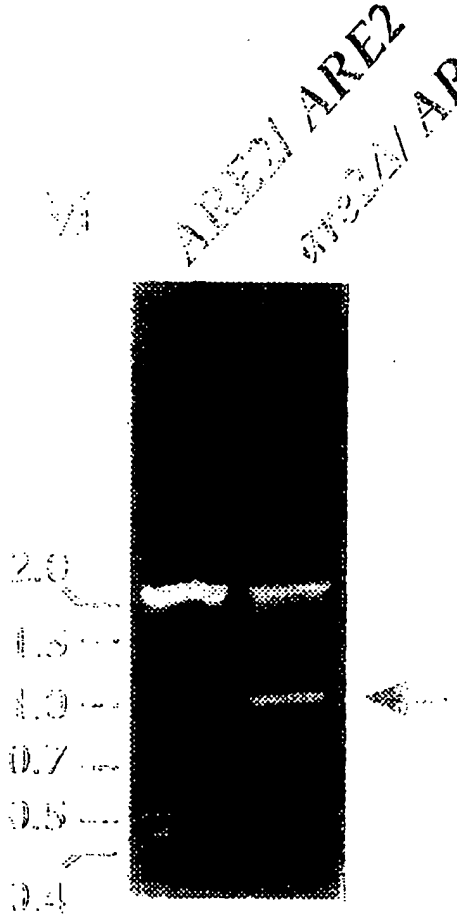


FIG. 3A
Wild-type

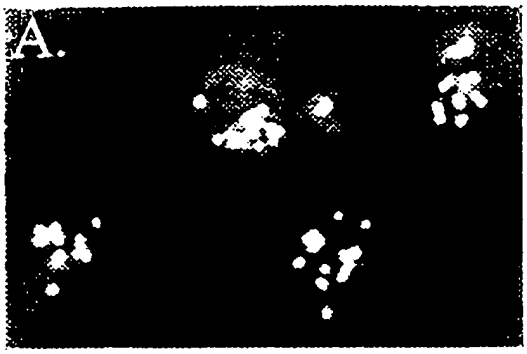


FIG. 3B
are1 are2 mutant

FIG. 4A
Triglyceride

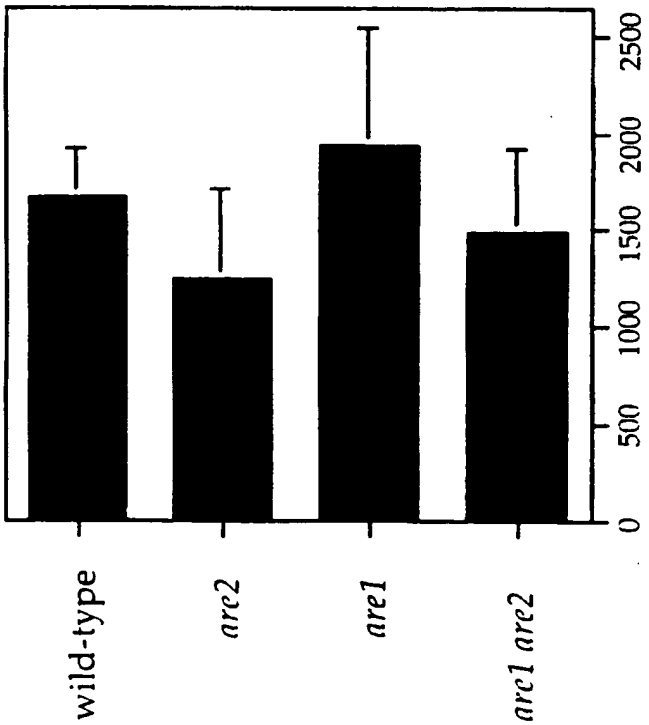


FIG. 4B
Sterol Ester

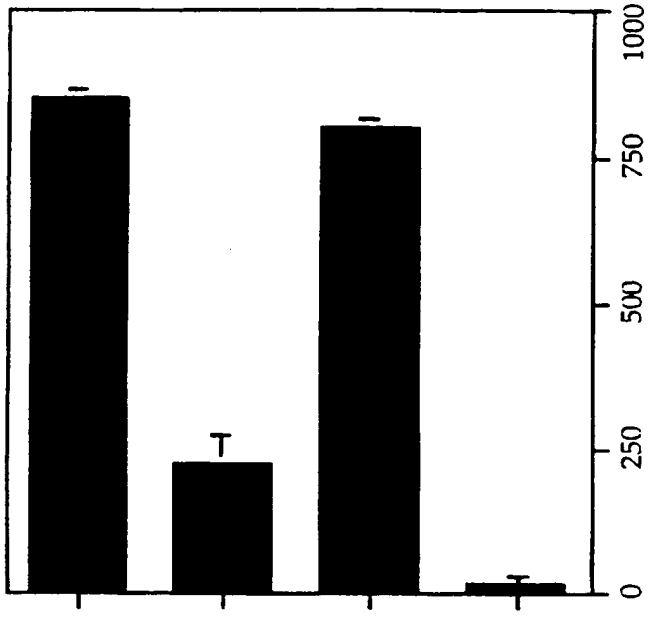
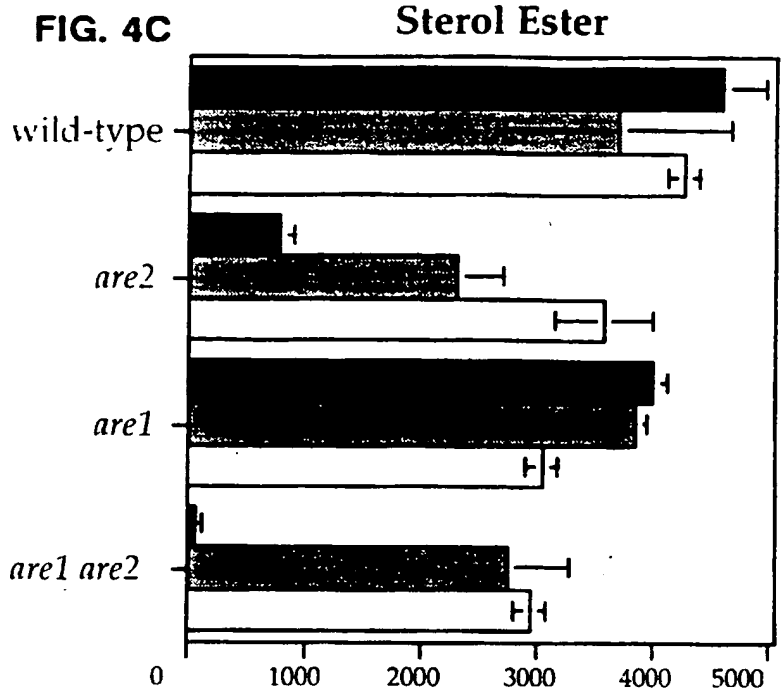
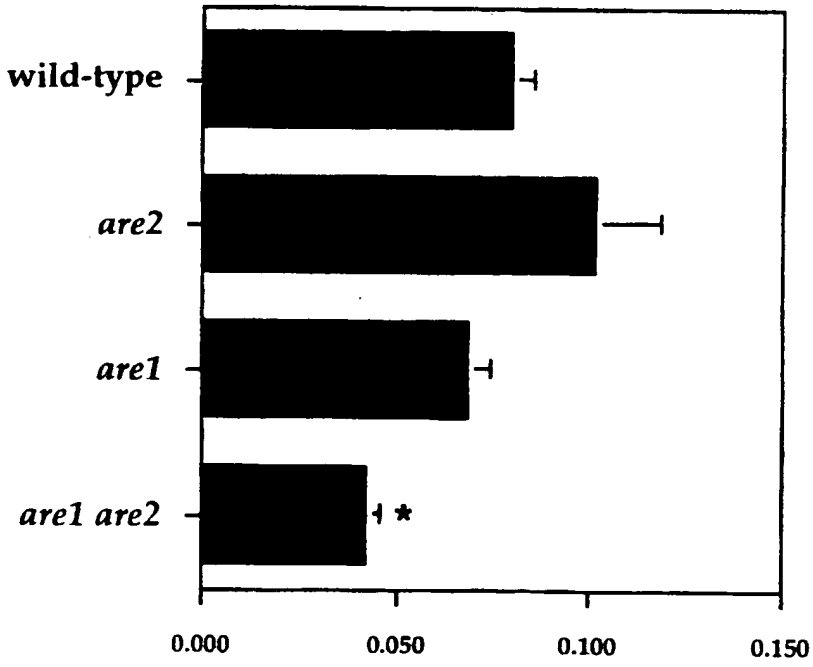


FIG. 4C



^{3}H-oleate incorporation
DPM/mg dry weight

FIG. 4D



^{14}C-acetate incorporation
(ratio of incorporation, sterol/fatty acid)

FIG. 5A-1

```
   1 gggtagagacggggtttcaccgtgttagccaggatggtctggatctcctgacctcgtgatccacccacctcggcctcctaaagtgctgggattacagaca 100
 101 tgagccaccgcgcccagccctattcatccctttcaaaagtcagaccctaggaagctggagggaggtggggcatggttttacagtgaatttctgatttca 200
 201 ctcagggtgataaatcagactcttggggaagcgggtggtggctctggacagcagcaggaatggggatccagttagcaacaaatccatggacctatgacag 300
 301 gctgaaagccacccctctccatctttgggaggttgccaatgtctgatttaacactatccaatgaatgatcattgaaagtaaaaaataactatcaactag 400
 401 cagaaaatataaatggtaagcattagcacatatttcacatgtttatatttggctctcagattgacctataaaacaaagtctgggaaattctatatgatcc 500
 501 tgaaaaaatgatacgctggtctggatggtagaataagttggagaaatgtttaagccaaaatgcagtcttaccaatgacttttattttattttattaatt 600
 601 ttcaggatttttggtatacaggtggtttttggttacatggaaaagttctttactggtgatttctgagattttagttcacccctatcctgagcagtgtac 700
 701 actgttcccaatatgtagccttttatccctcacccccctctaagttcaagaagactatggtcctgcagaaagctttatatgtaattaacatatctttatct 800
 801 ttatctttataggcagtagactcatcttttgaaacagattccattaagagtgaatgtgtaccctccctctagcctttattattactgtttttgctattac 900
 901 atgtgttagtgtatgtgaatttaatgcttaaaaatgtatcccattggctactatggcaaaaggttgactcataagagtttagcacgggttaagatctgaa 1000
1001 agttttctcccagcctcttatcactggcgcagacttcacaattcatggaagccaccagtgagatgacattgcctcaggcagttactatttttatattcta 1100
1101 taactcgaggagctcagggtttcggaaatcattaaacttttttgtccttttaaagttggagacagcaattgtagacagccttccagtgggttatctttt 1200
1201 tgtgtctccttacctgtggagaagcctattagctgggatatgtagttaaatagctatatttatatatatccagggcaccccgaattcgggagagcttccc 1300
1301 ggagtcgaccttcctgctggctgctctgtgaccgcttcccggctctgccctcttggccgaagtgcccgctgccgggcgcgggcctcagacaataca ATG 1399
   1                                                                                                   M   1

1400 GTG GGT GAA GAG AAG ATG TCT CTA AGA AAC CGG CTG TCA AAG TCC AGG GAA AAT CCT GAG GAA GAT GAA GAC CAG  1474
   2 V   G   E   E   K   M   S   L   R   N   R   L   S   K   S   R   E   N   P   E   E   D   E   D   Q    26

1475 AGA AAC CCT GCA AAG GAG TCC CTA GAG ACA CCT AGT AAT GGT CGA ATT GAC ATA AAA CAG TTG ATA GCA AAG AAG  1549
  27 R   N   P   A   K   E   S   L   E   T   P   S   N   G   R   I   D   I   K   Q   L   I   A   K   K    51

1550 ATA AAG TTG ACA GCA GAG GCA GAG GAA TTG AAG CCA TTT TTT ATG AAG GAA GTT GGC AGT CAC TTT GAT GAT TTT  1624
  52 I   K   L   T   A   E   A   E   E   L   K   P   F   F   M   K   E   V   G   S   H   F   D   D   F    76
```

FIG. 5A-2

```
1625 GTG ACC AAT CTC ATT GAA AAG TCA GCA TCA TTA GAT AAT GGT GGG TGC GCT CTC ACA ACC TTT TCT GTT CTT GAA 1699
  77 V   T   N   L   I   E   K   S   A   S   L   D   N   G   G   C   A   L   T   T   F   S   V   L   E 101

1700 GGA GAG AAA AAC AAC CAT AGA GCG AAG GAT TTG AGA GCA CCT CCA GAA CAA GGA AAG ATT TTT ATT GCA AGG CGC 1774
 102 G   E   K   N   N   H   R   A   K   D   L   R   A   P   P   E   Q   G   K   I   F   I   A   R   R 126

1775 TCT CTC TTA GAT GAA CTG CTT GAA GTG GAC CAC ATC AGA ACA ATA TAT CAC ATG TTT ATT GCC CTC CTC ATT CTC 1849
 127 S   L   L   D   E   L   L   E   V   D   H   I   R   T   I   Y   H   M   F   I   A   L   L   I   L 151

1850 TTT ATC CTC AGC ACA CTT GTA GTA GAT TAC ATT GAT GAA GGA AGG CTG GTG CTT GAG TTC AGC CTC CTG TCT TAT 1924
 152 F   I   L   S   T   L   V   V   D   Y   I   D   E   G   R   L   V   L   E   F   S   L   L   S   Y 176

1925 GCT TTT GGC AAA TTT CCT ACC GTT GTT TGG ACC TGG TGG ATC ATG TTC CTG TCT ACA TTT TCA GTT CCC TAT TTT 1999
 177 A   F   G   K   F   P   T   V   V   W   T   W   W   I   M   F   L   S   T   F   S   V   P   Y   F 201

2000 CTG TTT CAA CAT TGG CGC ACT GGC TAT AGC AAG AGT TCT CAT CCG CTG ATC CGT TCT CTC TTC CAT GGC TTT CTT 2074
 202 L   F   Q   H   W   R   T   G   Y   S   K   S   S   H   P   L   I   R   S   L   F   H   G   F   L 226

2075 TTC ATG ATC TTC CAG ATT GGA GTT CTA GGT TTT GGA CCA ACA TAT GTT GTG TTA GCA TAT ACA CTG CCA CCA GCT 2149
 227 F   M   I   F   Q   I   G   V   L   G   F   G   P   T   Y   V   V   L   A   Y   T   L   P   P   A 251

2150 TCC CGG TTC ATC ATT ATA TTC GAG CAG ATT CGT TTT GTA ATG AAG GCC CAC TCA TTT GTC AGA GAG AAC GTG CCT 2224
 252 S   R   F   I   I   I   F   E   Q   I   R   F   V   M   K   A   H   S   F   V   R   E   N   V   P 276

2225 CGG GTA CTA AAT TCA GCT AAG GAG AAA TCA AGC ACT GTT CCA ATA CCT ACA GTC AAC CAG TAT TTG TAC TTC TTA 2299
 277 R   V   L   N   S   A   K   E   K   S   S   T   V   P   I   P   T   V   N   Q   Y   L   Y   F   L 301

2300 TTT GCT CCT ACC CTT ATC TAC CGT GAC AGC TAT CCC AGG AAT CCC ACT GTA AGA TGG GGT TAT GTC GCT ATG AAG 2374
 302 F   A   P   T   L   I   Y   R   D   S   Y   P   R   N   P   T   V   R   W   G   Y   V   A   M   K 326

2375 TTT GCA CAG GTC TTT GGT TGC TTT TTC TAT GTG TAC TAC ATC TTT GAA AGG CTT TGT GCC CCC TTG TTT CGG AAT 2449
 327 F   A   Q   V   F   G   C   F   F   Y   V   Y   Y   I   F   E   R   L   C   A   P   L   F   R   N 351

2450 ATC AAA CAG GAG CCC TTC AGC GCT CGT GTT CTG GTC CTA TGT GTA TTT AAC TCC ATC TTG CCA GGT GTG CTG ATT 2524
 352 I   K   Q   E   P   F   S   A   R   V   L   V   L   C   V   F   N   S   I   L   P   G   V   L   I 376
```

FIG. 5A-3

```
2525 CTC TTC CTT ACT TTT TTT GCC TTT TTG CAC TGC TGG CTC AAT GCC TTT GCT GAG ATG TTA CGC TTT GGT GAC AGG  2599
 377 L   F   L   T   F   F   A   F   L   H   C   W   L   N   A   F   A   E   M   L   R   F   G   D   R    401

2600 ATG TTC TAT AAG GAT TGG TGG AAC TCC ACG TCA TAC TCC AAC TAT TAT AGA ACC TGG AAT GTG GTG GTC CAT GAC  2674
 402 M   F   Y   K   D   W   W   N   S   T   S   Y   S   N   Y   Y   R   T   W   N   V   V   V   H   D    426

2675 TGG CTA TAT TAC TAT GCT TAC AAG GAC TTT CTC TGG TTT TTC TCC AAG AGA TTC AAA TCT GCT GCC ATG TTA GCT  2749
 427 W   L   Y   Y   Y   A   Y   K   D   F   L   W   F   F   S   K   R   F   K   S   A   A   M   L   A    451

2750 GTC TTT GCT GTA TCT GCT GTA GTA CAC GAA TAT GCC TTG GCT GTT TGC TTG AGC TTT TTC TAT CCC GTG CTG TTC  2824
 452 V   F   A   V   S   A   V   V   H   E   Y   A   L   A   V   C   L   S   F   F   Y   P   V   L   F    476

2825 GTG CTC TTC ATG TTC TTT GGA ATG GCT TTC AAC TTC ATT GTC AAT GAT AGT CGG AAA AAG CCG ATT TGG AAT GTT  2899
 477 V   L   F   M   F   F   G   M   A   F   N   F   I   V   N   D   S   R   K   K   P   I   W   N   V    501

2900 CTG ATG TGG ACT TCT CTT TTC TTG GGC AAT GGA GTC TTA CTC TGC TTT TAT TCT CAA GAA TGG TAT GCA CGT CGG  2974
 502 L   M   W   T   S   L   F   L   G   N   G   V   L   L   C   F   Y   S   Q   E   W   Y   A   R   R    526

2975 CAC TGT CCT CTG AAA AAT CCC ACA TTT TTG GAT TAT GTC CGG CCA CGT TCC TGG ACT TGT CGT TAC GTG TTT TAG  3049
 527 H   C   P   L   K   N   P   T   F   L   D   Y   V   R   P   R   S   W   T   C   R   Y   V   F   *    551

3050 aagcttggactttgtttcctccttgtcactgaagattgggtagctccctgatttggagccagctgtttccagttgttactgaagttatctgtgttatttg 3149

3150 gaccactccaggctttacagatgactcactccattcctaggtcacttgaagccaaactgttggaagttcactggagtcttgtacacttaagcagagcaga 3249

3250 actttttttgtggggctgggtgggggggagaagaccgactaacagctgaagtaatgacagattgttgctgggtcatatcagctttatcccttggtaattat 3349

3350 atctgttttgtttcttgactctgtccaatcagagaataaacatcatagtttcttggccactgaattagccaaaacacttaggaagaaatcacttaaatac 3449

3450 ctctggcttagaaatttttcatgcacactgttggaatgtatgctaattgaacatgcaattggggaagaaaaaatgtagaatgatttttgctatttctag 3549

3550 tagaaagaaaatgtctgttttccaaagataatgttatacatcctattttgtaatttttttgaaaaaagttcaatgttcagttttccttagtttttacctt 3649
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FIG. 5B-1

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   1 gccctccagctctctactaagaccggtcgcaagcatgctgggcgatatatccaaaccacaccacacatggtctccctcctgcgtcaaaatctccccagac 100

 101 agtccggacccgcacccgatatccagaatgaaactgcacggctgcagattcaaaagctccaacgccctcagcgtcatcttcgcctggatatgctgcactc 200

 201 tggtcgaacccgtgtacttgtgtgcttcgctatcattatagaaaatctccggtggtgccaactcctcaggacgtgacattatttcttctctgatatattt 300

 301 cctgtgtttccgtaccgcacctttttagcactactttttttactatgctcttcttcttctgcttcttctgctttttttcctctttatcacactatgtatgtg 400

 401 ctgctcatctcttctttttatcgataaaattgaaaaatgtgagatggtgtagagtgaaaaaaaaaaaaaaaatctggcttggccatcaaatacccggccgt 500

 501 ggttggactcgtttagcgaacaatagcacccagcagaccctggcaacatgcggatgatataagaaggacgagcgtggtggaggaaaggggcgccattggc 600

 601 acactcacgcaggtggttgttcagcacggcttgcagcaagagcgccaaaacagattgcaaga ATG ACG GAG ACT AAG GAT TTG TTG CAA   689
   1                                                               M   T   E   T   K   D   L   L   Q     9

 690 GAC GAA GAG TTT CTT AAG ATC CGC AGA CTC AAT TCC GCA GAA GCC AAC AAA CGG CAT TCG GTC ACG TAC GAT AAC   764
  10 D   E   E   F   L   K   I   R   R   L   N   S   A   E   A   N   K   R   H   S   V   T   Y   D   N    34

 765 GTG ATC CTG CCA CAG GAG TCC ATG GAG GTT TCG CCA CGG TCG TCT ACC ACG TCG CTG GTG GAG CCA GTG GAG TCG   839
  35 V   I   L   P   Q   E   S   M   E   V   S   P   R   S   S   T   T   S   L   V   E   P   V   E   S    59

 840 ACT GAA GGA GTG GAG TCG ACT GAG GCG GAA CGT GTG GCA GGG AAG CAG GAG CAG GAG GAG GAG TAC CCT GTG GAC   914
  60 T   E   G   V   E   S   T   E   A   E   R   V   A   G   K   Q   E   Q   E   E   E   Y   P   V   D    84

 915 GCC CAC ATG CAA AAG TAC CTT TCA CAC CTG AAG AGC AAG TCT CGG TCG AGG TTC CAC CGA AAG GAT GCT AGC AAG   989
  85 A   H   M   Q   K   Y   L   S   H   L   K   S   K   S   R   S   R   F   H   R   K   D   A   S   K   109

 990 TAT GTG TCG TTT TTT GGG GAC GTG AGT TTT GAT CCT CGC CCC ACG CTC CTG GAC AGC GCC ATC AAC GTG CCC TTC  1064
 110 Y   V   S   F   F   G   D   V   S   F   D   P   R   P   T   L   L   D   S   A   I   N   V   P   F   134

1065 CAG ACG ACT TTC AAA GGT CCG GTG CTG GAG AAA CAG CTC AAA AAT TTA CAG TTG ACA AAG ACC AAG ACC AAG GCC  1139
 135 Q   T   T   F   K   G   P   V   L   E   K   Q   L   K   N   L   Q   L   T   K   T   K   T   K   A   159

1140 ACG GTG AAG ACT ACG GTG AAG ACT ACG GAG AAA ACG GAC AAG GCA GAT GCC CCC CCA GGA GAA AAA CTG GAG TCG  1214
 160 T   V   K   T   T   V   K   T   T   E   K   T   D   K   A   D   A   P   P   G   E   K   L   E   S   184

1215 AAC TTT TCA GGG ATC TAC GTG TTC GCA TGG ATG TTC TTG GGC TGG ATA GCC ATC AGG TGC TGC ACA GAT TAC TAT  1289
 185 N   F   S   G   I   Y   V   F   A   W   M   F   L   G   W   I   A   I   R   C   C   T   D   Y   Y   209
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FIG. 5B-2

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1290 GCG TCG TAC GGC AGT GCA TGG AAT AAG CTG GAA ATC GTG CAG TAC ATG ACA ACG GAC TTG TTC ACG ATC GCA ATG 1364
 210 A   S   Y   G   S   A   W   N   K   L   E   I   V   Q   Y   M   T   T   D   L   F   T   I   A   M   234

1365 TTG GAC TTG GCA ATG TTC CTG TGC ACT TTC TTC GTG GTT TTC GTG CAC TGG CTG GTG AAA AAG CGG ATC ATC AAC 1439
 235 L   D   L   A   M   F   L   C   T   F   F   V   V   F   V   H   W   L   V   K   K   R   I   I   N   259

1440 TGG AAG TGG ACT GGG TTC GTT GCA GTG AGC ATC TTC GAG TTG GCT TTC ATC CCC GTG ACG TTC CCC ATT TAC GTC 1514
 260 W   K   W   T   G   F   V   A   V   S   I   F   E   L   A   F   I   P   V   T   F   P   I   Y   V   284

1515 TAC TAC TTT GAT TTC AAC TGG GTC ACG AGA ATC TTC CTG TTC CTG CAC TCC GTG GTG TTT GTT ATG AAG AGC CAC 1589
 285 Y   Y   F   D   F   N   W   V   T   R   I   F   L   F   L   H   S   V   V   F   V   M   K   S   H   309

1590 TCG TTT GCC TTT TAC AAC GGG TAT CTT TGG GAC ATA AAG CAG GAA CTC GAG TAC TCT TCC AAA CAG TTG CAA AAA 1664
 310 S   F   A   F   Y   N   G   Y   L   W   D   I   K   Q   E   L   E   Y   S   S   K   Q   L   Q   K   334

1665 TAC AAG GAA TCT TTG TCC CCA GAG ACC CGC GAG ATT CTG CAA AAA AGT TGC GAC TTT TGC CTT TTC GAA TTG AAC 1739
 335 Y   K   E   S   L   S   P   E   T   R   E   I   L   Q   K   S   C   D   F   C   L   F   E   L   N   359

1740 TAC CAG ACC AAG GAT AAC GAC TTC CCC AAC AAC ATC AGT TGC AGC AAT TTC TTC ATG TTC TGT TTG TTC CCC GTC 1814
 360 Y   Q   T   K   D   N   D   F   P   N   N   I   S   C   S   N   F   F   M   F   C   L   F   P   V   384

1815 CTC GTG TAC CAG ATC AAC TAC CCA AGA ACG TCG CGC ATC AGA TGG AGG TAT GTG TTG GAG AAG GTG TGC GCC ATC 1889
 385 L   V   Y   Q   I   N   Y   P   R   T   S   R   I   R   W   R   Y   V   L   E   K   V   C   A   I   409

1890 ATT GGC ACC ATC TTC CTC ATG ATG GTC ACG GCA CAG TTC TTC ATG CAC CCG GTG GCC ATG CGC TGT ATC CAG TTC 1964
 410 I   G   T   I   F   L   M   M   V   T   A   Q   F   F   M   H   P   V   A   M   R   C   I   Q   F   434

1965 CAC AAC ACG CCC ACC TTC GGC GGC TGG ATC CCC GCC ACG CAA GAG TGG TTC CAC CTG CTC TTC GAC ATG ATT CCG 2039
 435 H   N   T   P   T   F   G   G   W   I   P   A   T   Q   E   W   F   H   L   L   F   D   M   I   P   459

2040 GGC TTC ACT GTT CTG TAC ATG CTC ACG TTT TAC ATG ATA TGG GAC GCT TTA TTG AAT TGC GTG GCG GAG TTG ACC 2114
 460 G   F   T   V   L   Y   M   L   T   F   Y   M   I   W   D   A   L   L   N   C   V   A   E   L   T   484
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FIG. 5B-3

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2115 AGG TTT GCG GAC AGA TAT TTC TAC GGC GAC TGG TGG AAT TGC GTT TCG TTT GAA GAG TTT AGC AGA ATC TGG AAC 2189
 485 R   F   A   D   R   Y   F   Y   G   D   W   W   N   C   V   S   F   E   E   F   S   R   I   W   N   509

2190 GTC CCC GTT CAC AAA TTT TTA CTA AGA CAC GTG TAC CAC AGC TCC ATG GGC GCA TTG CAT TTG AGC AAG AGC CAA 2264
 510 V   P   V   H   K   F   L   L   R   H   V   Y   H   S   S   M   G   A   L   H   L   S   K   S   Q   534

2265 GCT ACA TTA TTT ACT TTT TTC TTG AGT GCC GTG TTC CAC GAA ATG GCC ATG TTC GCC ATT TTC AGA AGG GTT AGA 2339
 535 A   T   L   F   T   F   F   L   S   A   V   F   H   E   M   A   M   F   A   I   F   R   R   V   R   559

2340 GGA TAT CTG TTC ATG TTC CAA CTG TCG CAG TTT GTG TGG ACT GCT TTG AGC AAC ACC AAG TTT CTA CGG GCA AGA 2414
 560 G   Y   L   F   M   F   Q   L   S   Q   F   V   W   T   A   L   S   N   T   K   F   L   R   A   R   584

2415 CCG CAG TTG TCC AAC GTT GTC TTT TCG TTT GGT GTC TGT TCA GGG CCC AGT ATC ATT ATG ACG TTG TAC CTG ACC 2489
 585 P   Q   L   S   N   V   V   F   S   F   G   V   C   S   G   P   S   I   I   M   T   L   Y   L   T   609

2490 TTA TGA actgccaccataccacgtgtgtccctcgcaagcccttgatagatatacaatagggaatgggcgtccgtccaccgtggtcaaagacaggggca 2587
 610 L   *                                                                                               611

2588 aagagctcctaggt                                                                                      2601
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FIG. 5C-1

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  1 tataaaattccttttcatcaatacatctatatattcgaatatatagataaaccaatacaaaaacatactgaaatttttgaaaacaactaaaactattcat 100

101 tgcagttacacgtgaatgctaaactttatatcgctcttgtcggtcccgcggagttaacatttaacggcttctcgcgcaataaccggaaaaattccaacag 200

201 tttctttgtaatattattaagccttctttttttcccggaatctataagaggggacgaaaattagccgctattaattctggtattgccacctagacaagaag 300

301 taaacagacacattacgttagcaaaagcaacaataacaaacacaacc ATG GAC AAG AAG AAG GAT CTA CTG GAG AAC GAA CAA TTT  386
  1                                               M   D   K   K   K   D   L   L   E   N   E   Q   F    13

387 CTC CGC ATC CAA AAG CTC AAC GCT GCC GAT GCG GGC AAA AGA CAA TCT ATA ACA GTG GAC GAC GAG GGC GAA CTA  461
 14 L   R   I   Q   K   L   N   A   A   D   A   G   K   R   Q   S   I   T   V   D   D   E   G   E   L    38

462 TAT GGG TTA GAC ACC TCC GGC AAC TCA CCA GCC AAT GAA CAC ACA GCT ACC ACA ATT ACA CAG AAT CAC AGC GTG  536
 39 Y   G   L   D   T   S   G   N   S   P   A   N   E   H   T   A   T   T   I   T   Q   N   H   S   V    63

537 GTG GCC TCA AAC GGA GAC GTC GCA TTC ATC CCA GGA ACT GCT ACC GAA GGC AAT ACA GAG ATT GTA ACT GAA GAA  611
 64 V   A   S   N   G   D   V   A   F   I   P   G   T   A   T   E   G   N   T   E   I   V   T   E   E    88

612 GTG ATT GAG ACC GAT GAT AAC ATG TTC AAG ACC CAT GTG AAG ACT TTA AGC TCC AAA GAG AAG GCA CGG TAT AGG  686
 89 V   I   E   T   D   D   N   M   F   K   T   H   V   K   T   L   S   S   K   E   K   A   R   Y   R   113

687 CAA GGG TCC TCT AAC TTT ATA TCG TAT TTC GAT GAT ATG TCA TTT GAA CAC AGG CCC AGT ATA TTA GAT GGG TCA  761
114 Q   G   S   S   N   F   I   S   Y   F   D   D   M   S   F   E   H   R   P   S   I   L   D   G   S   138

762 GTT AAC GAG CCC TTC AAG ACC AAA TTC GTG GGA CCT ACT TTA GAA AAG GAG ATC AGA AGA AGG GAG AAA GAG CTA  836
139 V   N   E   P   F   K   T   K   F   V   G   P   T   L   E   K   E   I   R   R   R   E   K   E   L   163

837 ATG GCC ATG CGC AAA AAT TTA CAC CAC CGC AAG TCC TCC CCA GAT GCT GTC GAC TCA GTA GGG AAA AAT GAT GGC  911
164 M   A   M   R   K   N   L   H   H   R   K   S   S   P   D   A   V   D   S   V   G   K   N   D   G   188

912 GCC GCC CCA ACT ACT GTT CCA ACT GCC GCC ACC TCA GAA ACG GTG GTC ACC GTT GAA ACC ACC ATA ATT TCA TCC  986
189 A   A   P   T   T   V   P   T   A   A   T   S   E   T   V   V   T   V   E   T   T   I   I   S   S   213

987 AAT TTC TCC GGG TTG TAC GTG GCG TTT TGG ATG GCT ATT GCA TTT GGT GCT GTC AAG GCT TTA ATA GAC TAT TAT 1061
214 N   F   S   G   L   Y   V   A   F   W   M   A   I   A   F   G   A   V   K   A   L   I   D   Y   Y   238
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FIG. 5C-2

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1062 TAC CAG CAT AAT GGT AGC TTC AAG GAT TCG GAG ATC TTG AAA TTT ATG ACT ACG AAT TTG TTC ACT GTG GCA TCC 1136
 239 Y   Q   H   N   G   S   F   K   D   S   E   I   L   K   F   M   T   T   N   L   F   T   V   A   S   263

1137 GTA GAT CTT TTG ATG TAT TTG AGC ACT TAT TTT GTC GTT GGA ATA CAA TAC TTA TGC AAG TGG GGG GTC TTG AAA 1211
 264 V   D   L   L   M   Y   L   S   T   Y   F   V   V   G   I   Q   Y   L   C   K   W   G   V   L   K   288

1212 TGG GGC ACT ACC GGC TGG ATC TTC ACC TCA ATT TAC GAG TTT TTG TTT GTT ATC TTC TAC ATG TAT TTA ACA GAA 1286
 289 W   G   T   T   G   W   I   F   T   S   I   Y   E   F   L   F   V   I   F   Y   M   Y   L   T   E   313

1287 AAC ATC CTA AAA CTA CAC TGG CTG TCC AAG ATC TTC CTT TTT TTG CAT TCT TTA GTT TTA TTG ATG AAA ATG CAT 1361
 314 N   I   L   K   L   H   W   L   S   K   I   F   L   F   L   H   S   L   V   L   L   M   K   M   H   338

1362 TCT TTC GCC TTC TAC AAT GGC TAT CTA TGG GGT ATA AAG GAA GAA CTA CAA TTT TCC AAA AGC GCT CTT GCC AAA 1436
 339 S   F   A   F   Y   N   G   Y   L   W   G   I   K   E   E   L   Q   F   S   K   S   A   L   A   K   363

1437 TAC AAG GAT TCT ATA AAT GAT CCA AAA GTT ATT GGT GCT CTT GAG AAA AGC TGT GAG TTT TGT AGT TTT GAA TTG 1511
 364 Y   K   D   S   I   N   D   P   K   V   I   G   A   L   E   K   S   C   E   F   C   S   F   E   L   388

1512 AGC TCT CAG TCT TTA AGC GAC CAA ACT CAA AAA TTC CCC AAC AAT ATC AGT GCA AAA AGC TTT TTT TGG TTC ACC 1586
 389 S   S   Q   S   L   S   D   Q   T   Q   K   F   P   N   N   I   S   A   K   S   F   F   W   F   T   413

1587 ATG TTT CCA ACC CTA ATT TAC CAA ATT GAA TAT CCA AGA ACT AAG GAA ATC AGA TGG AGC TAC GTA TTA GAA AAG 1661
 414 M   F   P   T   L   I   Y   Q   I   E   Y   P   R   T   K   E   I   R   W   S   Y   V   L   E   K   438

1662 ATC TGC GCC ATC TTC GGT ACC ATT TTC TTA ATG ATG ATA GAT GCT CAA ATC TTG ATG TAT CCT GTA GCA ATG AGA 1736
 439 I   C   A   I   F   G   T   I   F   L   M   M   I   D   A   Q   I   L   M   Y   P   V   A   M   R   463

1737 GCA TTG GCT GTG CGC AAT TCT GAA TGG ACT GGT ATA TTG GAT AGA TTA TTG AAA TGG GTT GGA TTG CTC GTT GAT 1811
 464 A   L   A   V   R   N   S   E   W   T   G   I   L   D   R   L   L   K   W   V   G   L   L   V   D   488

1812 ATC GTC CCA GGG TTT ATC GTG ATG TAC ATC TTG GAC TTC TAT TTG ATT TGG GAT GCC ATT TTG AAC TGT GTG GCT 1886
 489 I   V   P   G   F   I   V   M   Y   I   L   D   F   Y   L   I   W   D   A   I   L   N   C   V   A   513

1887 GAA TTG ACA AGA TTT GGC GAC AGA TAT TTC TAC GGT GAC TGG TGG AAT TGT GTT AGT TGG GCA GAC TTC AGT AGA 1961
 514 E   L   T   R   F   G   D   R   Y   F   Y   G   D   W   W   N   C   V   S   W   A   D   F   S   R   538
```

FIG. 5C-3

```
1962 ATT TGG AAC ATC CCA GTG CAT AAG TTT TTG TTA AGA CAT GTT TAC CAT AGT TCA ATG AGT TCA TTC AAA TTG AAC 2036
 539 I   W   N   I   P   V   H   K   F   L   L   R   H   V   Y   H   S   S   M   S   S   F   K   L   N  563

2037 AAG AGT CAA GCA ACT TTG ATG ACC TTT TTC TTA AGT TCC GTC GTT CAT GAA TTA GCA ATG TAC GTT ATC TTC AAG 2111
 564 K   S   Q   A   T   L   M   T   F   F   L   S   S   V   V   H   E   L   A   M   Y   V   I   F   K  588

2112 AAA TTG AGG TTT TAC TTG TTC TTC TTC CAA ATG CTG CAA ATG CCA TTA GTA GCT TTA ACA AAT ACT AAA TTC ATG 2186
 589 K   L   R   F   Y   L   F   F   F   Q   M   L   Q   M   P   L   V   A   L   T   N   T   K   F   M  613

2187 AGG AAC AGA ACC ATA ATC GGA AAT GTT ATT TTC TGG CTC GGT ATC TGC ATG GGA CCA AGT GTC ATG TGT ACG TTG 2261
 614 R   N   R   T   I   I   G   N   V   I   F   W   L   G   I   C   M   G   P   S   V   M   C   T   L  638

2262 TAC TTG ACA TTC TAA ggcatcctgcaactgttctgtggagctattaaatctttatagtaaatttttttttacttttttttttttttttttttt 2356
 639 Y   L   T   F   *                                                                                  643

2357 tttattatttacaagcgtctatatattttctattatagaatattgtcatttattacattggttca                                  2421
```

FIG. 5D

```
  1 ATG GAC AAC GCG GGG TCT GAT ACG ACT CAC TAT AGG GAA TTT GGC CCT CGA GCA GTA GAT TCG GCA CGA TGG GCA  75
  1 M   D   N   A   G   S   D   T   T   H   Y   R   E   F   G   P   R   A   V   D   S   A   R   W   A   25

 76 CGA GGA CTC CAT CAT GTT CCT CAA GCT TTA TTC CTA CCG GGA TGT CAA CCT GTG GTG CCG CCA GCG AAG GGT CAA 150
 26 R   G   L   H   H   V   P   Q   A   L   F   L   P   G   C   Q   P   V   V   P   P   A   K   G   Q   50

151 GGC CAA AGC TGT CTC TAC AGG GAA GAA GGT CAG TGG GGC TGC TGC GAG CAA GCT GTG AGC TAT CCA GAC AAC CTG 225
 51 G   Q   S   C   L   Y   R   E   E   G   Q   W   G   C   C   E   Q   A   V   S   Y   P   D   N   L   75

226 ACC TAC CGA GAT CTC GAT TAC TTC ATC TTT GCT CCT ACT TTG TGT TAT GAA CTC AAC TTT CCT CGG TCC CCC CGA 300
 76 T   Y   R   D   L   D   Y   F   I   F   A   P   T   L   C   Y   E   L   N   F   P   R   S   P   R  100

301 ATA CGA GAG CGC TTT CTG CTA CGA CGA GTT CTT GAG ATG CTC TTT TTT ACC CAG CTT CAA GTG GGG CTG ATC CAA 375
101 I   R   E   R   F   L   L   R   R   V   L   E   M   L   F   F   T   Q   L   Q   V   G   L   I   Q  125

376 CAG TGG ATG GTC CCT ACT ATC CAG AAC TCC ATG GAA GCC CTT TCA AGA GCT TCT GGC AGT TTT GGA GAC CGC GAG 450
126 Q   W   M   V   P   T   I   Q   N   S   M   E   A   L   S   R   A   S   G   S   F   G   D   R   E  150

451 TTC TAC AGA GAT TGG TGG AAT GCT GAG TCT GTC ACC GAC TTT TGG CAG AAC TGG AAT ATC CCC GTG G          517
151 F   Y   R   D   W   W   N   A   E   S   V   T   D   F   W   Q   N   W   N   I   P   V              172
```

FIG. 6A

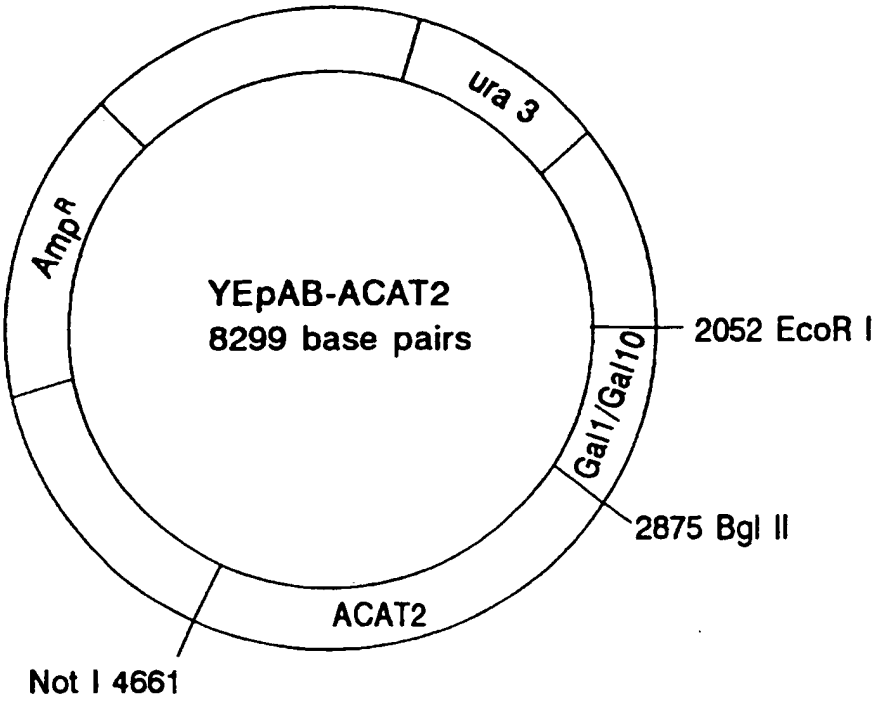

ura 3
Amp^R
YEpAB-ACAT2
8299 base pairs
2052 EcoR I
Gal1/Gal10
2875 Bgl II
ACAT2
Not I 4661

FIG. 6B

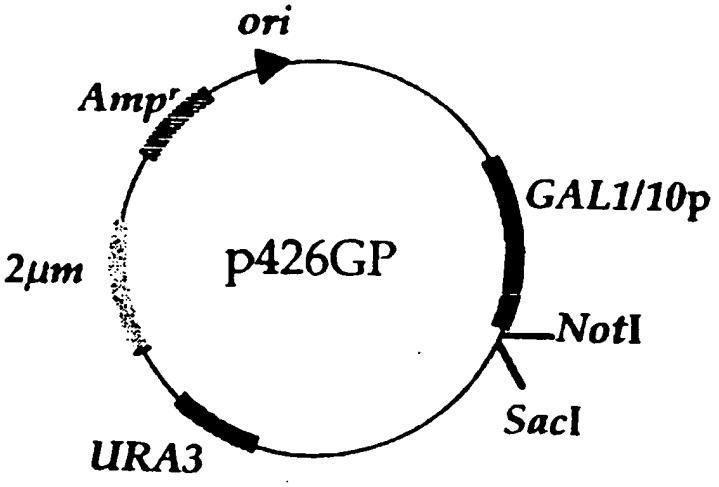
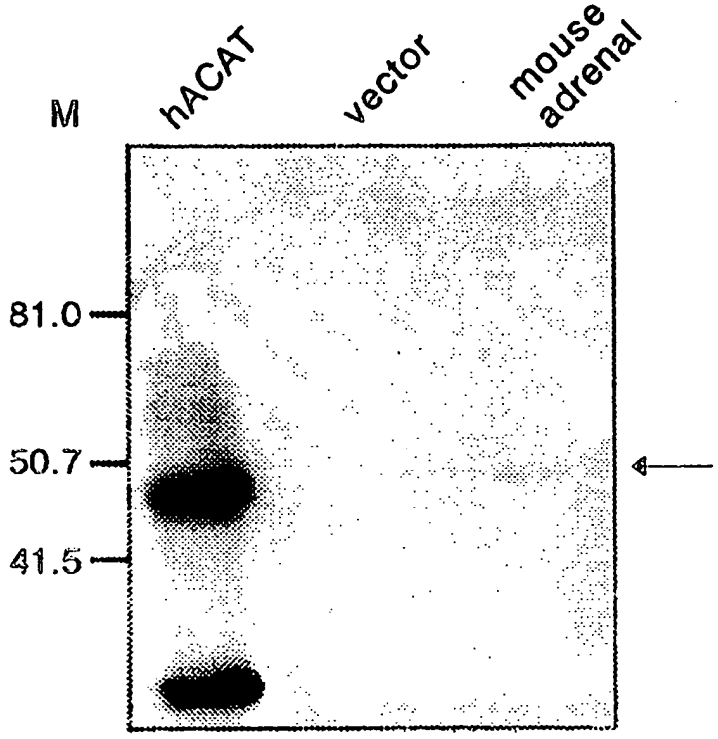

ori
Amp^r
GAL1/10p
2μm
p426GP
NotI
SacI
URA3

FIG. 6C



α hACAT, DM10

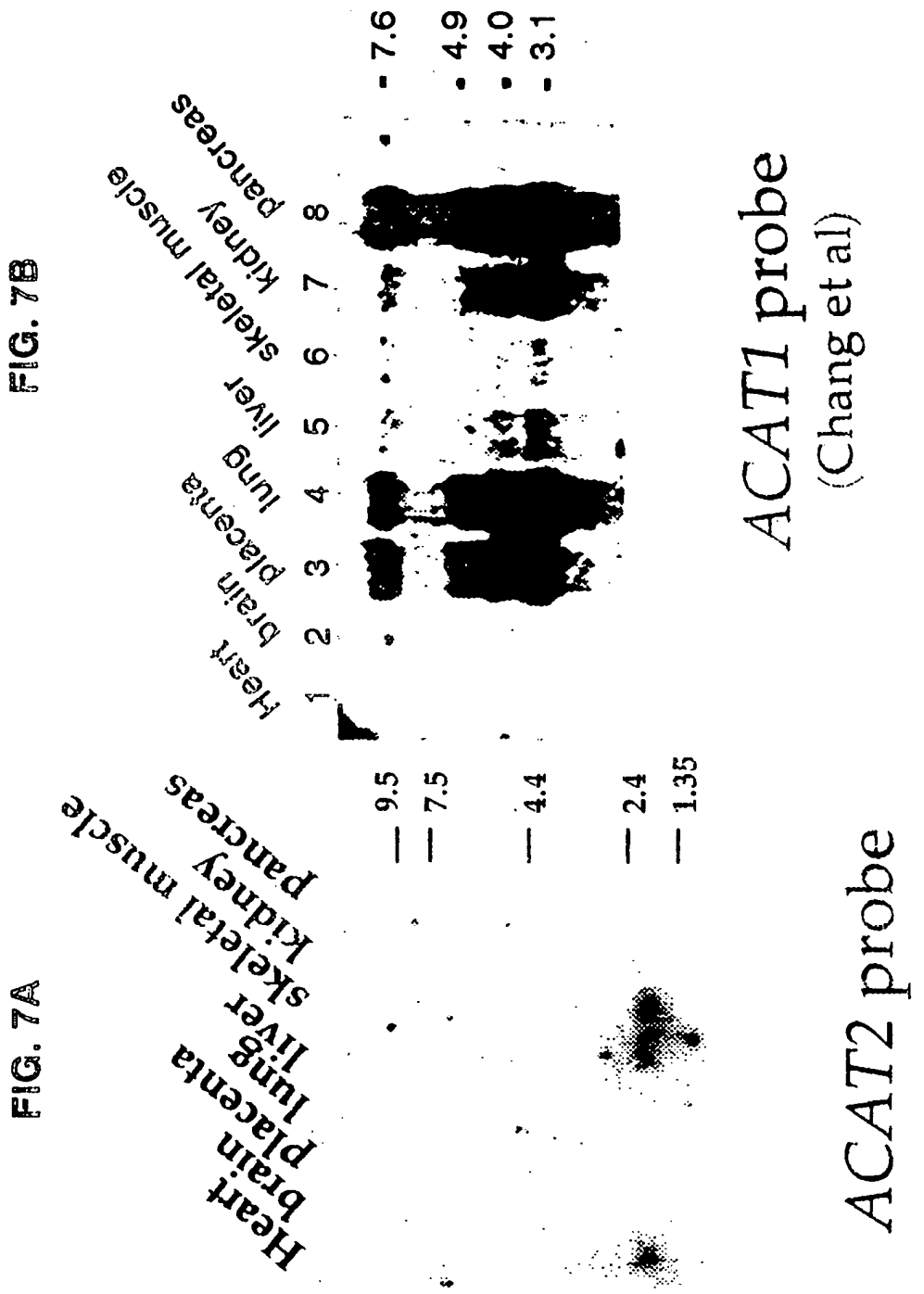


FIG. 7A
Heart
brain
placenta
lung
liver
skeletal muscle
kidney
pancreas
9.5
7.5
4.4
2.4
1.35
ACAT2 probe
FIG. 7B
1 2 3 4 5 6 7 8
Heart
brain
placenta
lung
liver
skeletal muscle
kidney
pancreas
7.6
4.9
4.0
3.1
ACAT1 probe
(Chang et al)

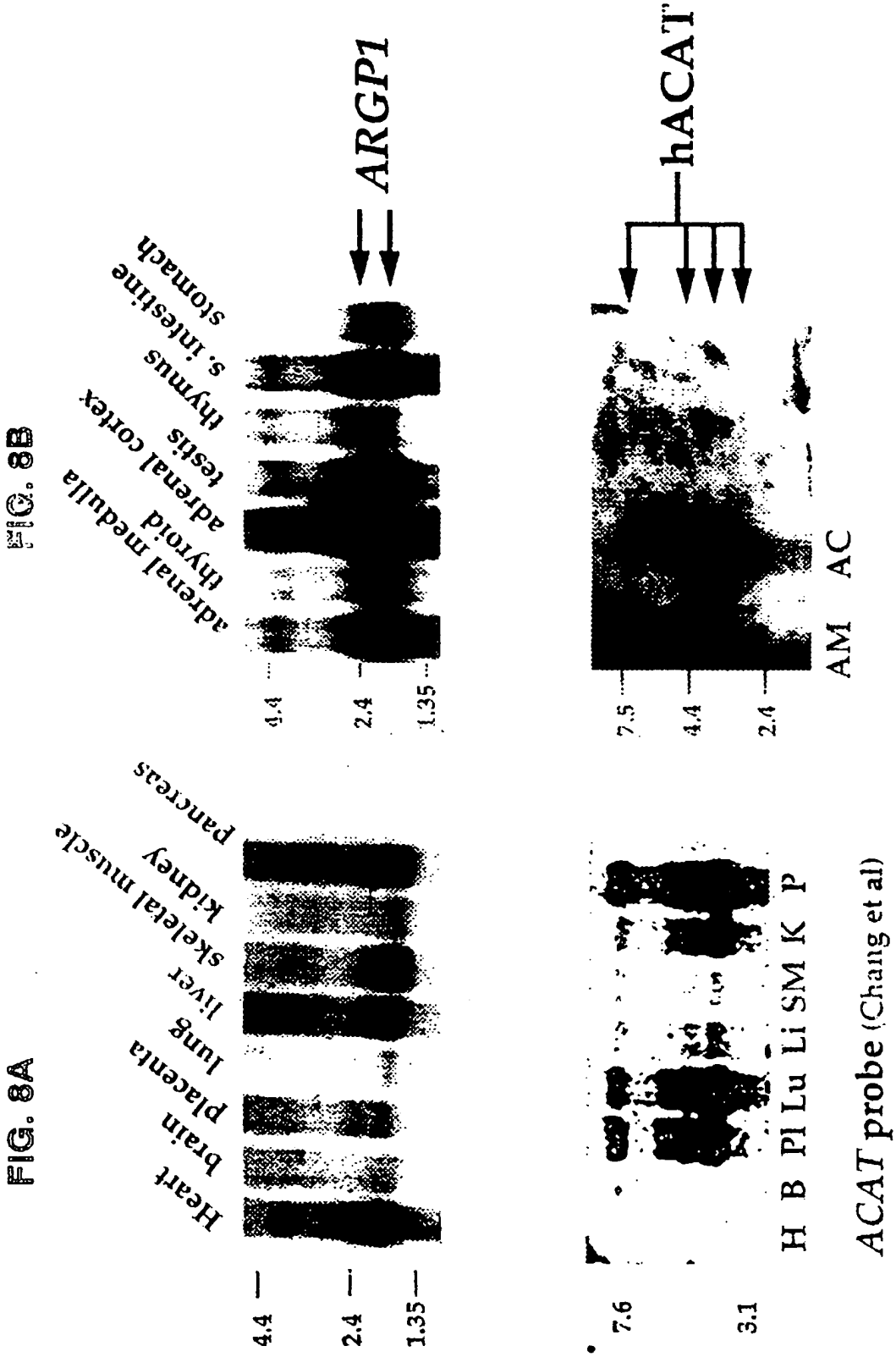


FIG. 8A
Heart
Brain
Placenta
Lung
Liver
Skeletal muscle
Kidney
Pancreas
4.4
2.4
1.35
FIG. 8B
ARGP1
adrenal medulla
thyroid
adrenal cortex
testis
thymus
s. intestine
stomach
4.4
2.4
1.35
FIG. 8C
ACAT probe (Chang et al)
H B Pl Lu Li SM K P
7.6
3.1
FIG. 8D
hACAT
AM AC
7.5
4.4
2.4

FIG. 9

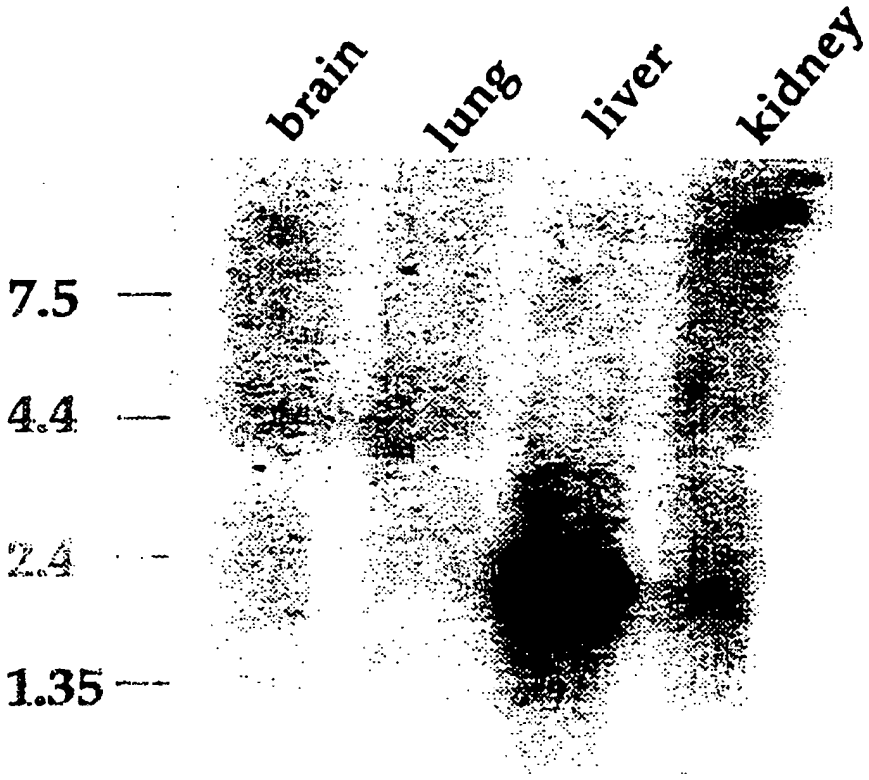


FIG. 10

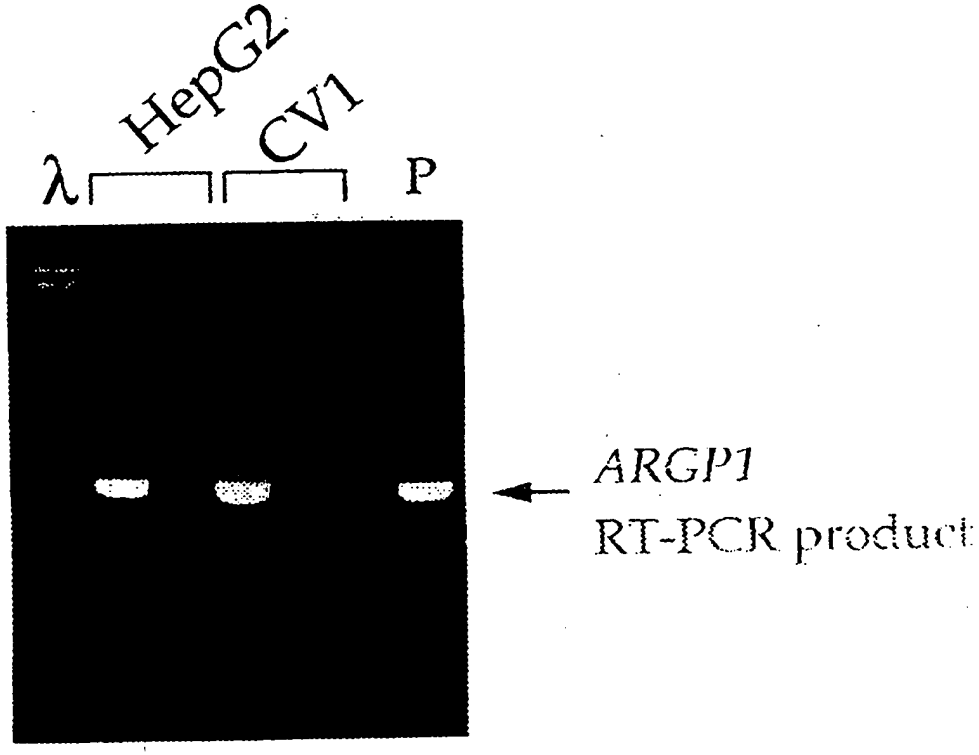


FIG. 11

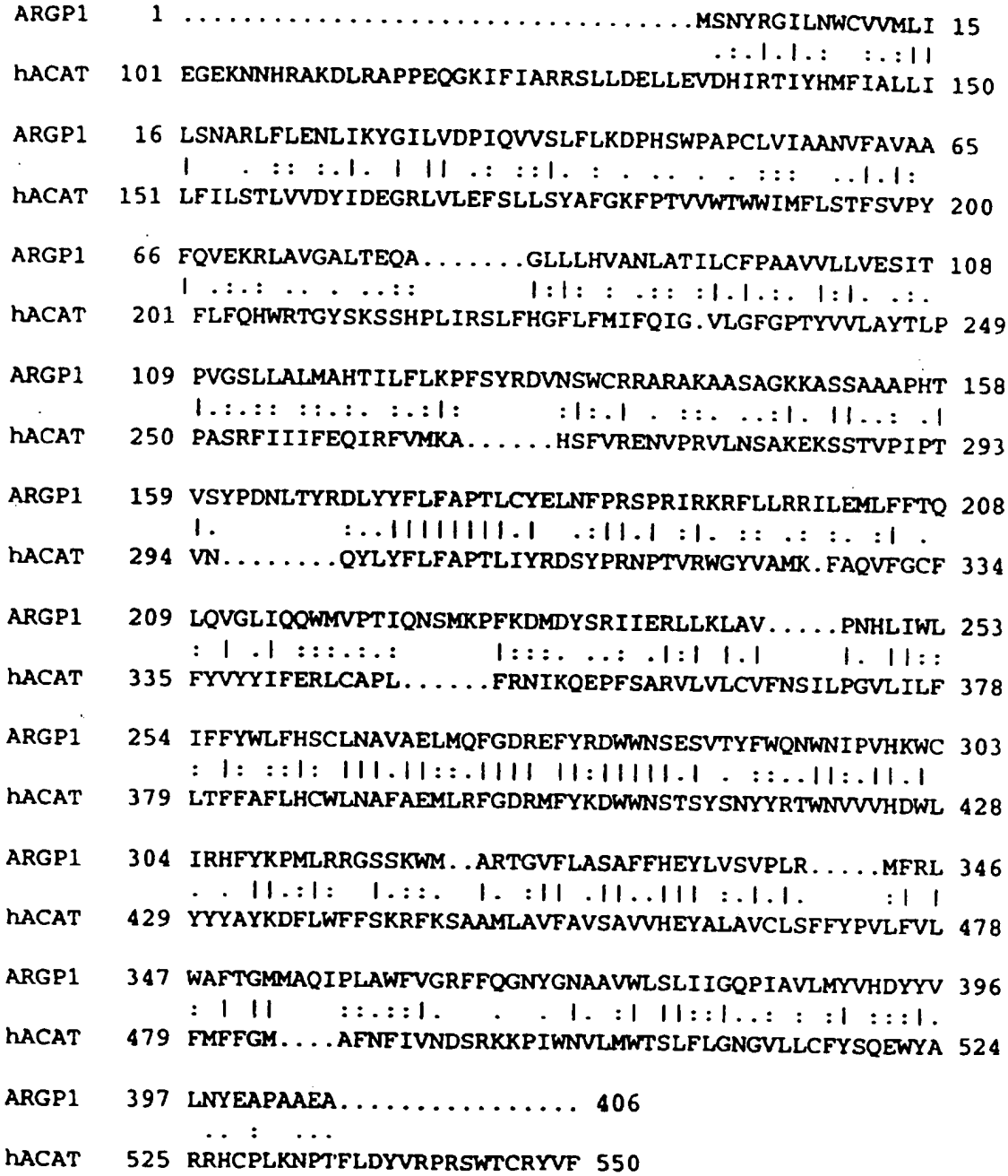
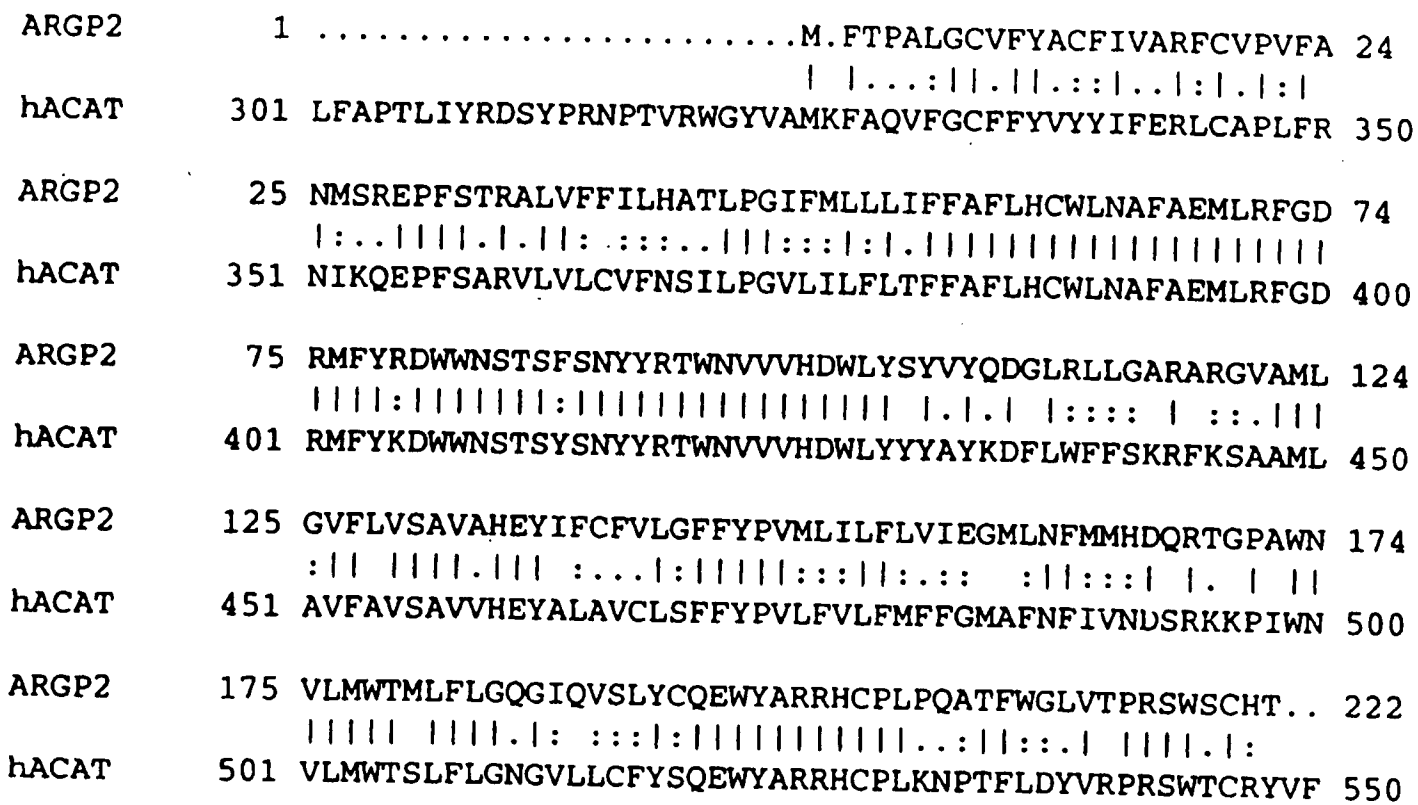

ARGP1 1 MSNYRGILNWCVVMLI 15
hACAT 101 EGEKNNHRAKDLRAPPEQGKIFIARRSLLDELLEVDHIRTIYHMFIALLI 150
ARGP1 16 LSNARLFLENLIKYGILVDPIQVVSLFLKDPHSWPAPCLVIAANVFAVAA 65
hACAT 151 LFILSTLVVDYIDEGRLVLEFSLLSYAFGKFPTVVWTWWIMFLSTFSVPY 200
ARGP1 66 FQVEKRLAVGALTEQA.....GLLLHVANLATILCFPAAVVLLVESIT 108
hACAT 201 FLFQHWRTGYSKSSHPLIRSLFHGFLFMIFQIG.VLGFGPTYVVLAYTLP 249
ARGP1 109 PVGSLLALMAHTILFLKPFSYRDVNSWCRRARAKAASAGKKASSAAAPHT 158
hACAT 250 PASRFIIIFEQIRFVMKA.....HSFVRENVPRVLNSAKEKSSTVPIPT 293
ARGP1 159 VSYPDNLTYRDLYYFLFAPTLCYELNFPRSPRIRKRFLLRRILEMLFFTQ 208
hACAT 294 VN.....QYLYFLFAPTLIYRDSYPRNPTVRWGYVAMK.FAQVFGCF 334
ARGP1 209 LQVGLIQQWMVPTIQNSMKPFKDMDYSRIIERLLKLAV.....PNHLIWL 253
hACAT 335 FYVYYIFERLCAPL.....FRNIKQEPFSARVLVLCVFNSILPGVLILF 378
ARGP1 254 IFFYWLFHSCLNAVAELMQFGDREFYRDWWNSESVTYFWQNWNIPVHKWC 303
hACAT 379 LTFFAFLHCWLNAFAEMLRFGDRMFYKDWWNSTSYSNYYRTWNVVVHDWL 428
ARGP1 304 IRHFYKPMLRRGSSKWM..ARTGVFLASAFFHEYLVSVPLR.....MFRL 346
hACAT 429 YYYAYKDFLWFFSKRFKSAAMLAVFAVSAVVHEYALAVCLSFFYPVLFVL 478
ARGP1 347 WAFTGMMAQIPLAWFVGRFFQGNYGNAAVWLSLIIGQPIAVLMYVHDYYV 396
hACAT 479 FMFFGM....AFNFIVNDSRKKPIWNVLMWTSLFLGNGVLLCFYSQEWYA 524
ARGP1 397 LNYEAPAAEA..... 406
hACAT 525 RRHCPLKNPTFLDYVRPRSWTCRYVF 550

FIG. 12


ARGP2 1 .....M.FTPALGCVFYACFIVARFCVPVFA 24
hACAT 301 LFAPTLIYRDSYPRNPTVRWGYVAMKFAQVFGCFFYVYYIFERLCAPLFR 350
ARGP2 25 NMSREPFSTRALVFFILHATLPGIFMLLLIFFAFLHCWLNAFAEMLRFGD 74
hACAT 351 NIKQEPFSARVLVLCVFNSILPGVLILFLTFFAFLHCWLNAFAEMLRFGD 400
ARGP2 75 RMFYRDWWNSTSFSNYYRTWNVVVHDWLYSYVYQDGLRLLGARARGVAML 124
hACAT 401 RMFYKDWWNSTSYSNYYRTWNVVVHDWLYYYAYKDFLWFFSKRFKSAAML 450
ARGP2 125 GVFLVSAVAHEYIFCFVLGFFYPVMLILFLVIEGMLNFMMHDQRTGPAWN 174
hACAT 451 AVFAVSAVVHEYALAVCLSFFYPVLFVLFMFFGMAFNFIVNDSRKKPIWN 500
ARGP2 175 VLMWTMLFLGQGIQVSLYCQEWYARRHCPLPQATFWGLVTPRSWSCHT.. 222
hACAT 501 VLMWTSLFLGNGVLLCFYSQEWYARRHCPLKNPTFLDYVRPRSWTCRYVF 550

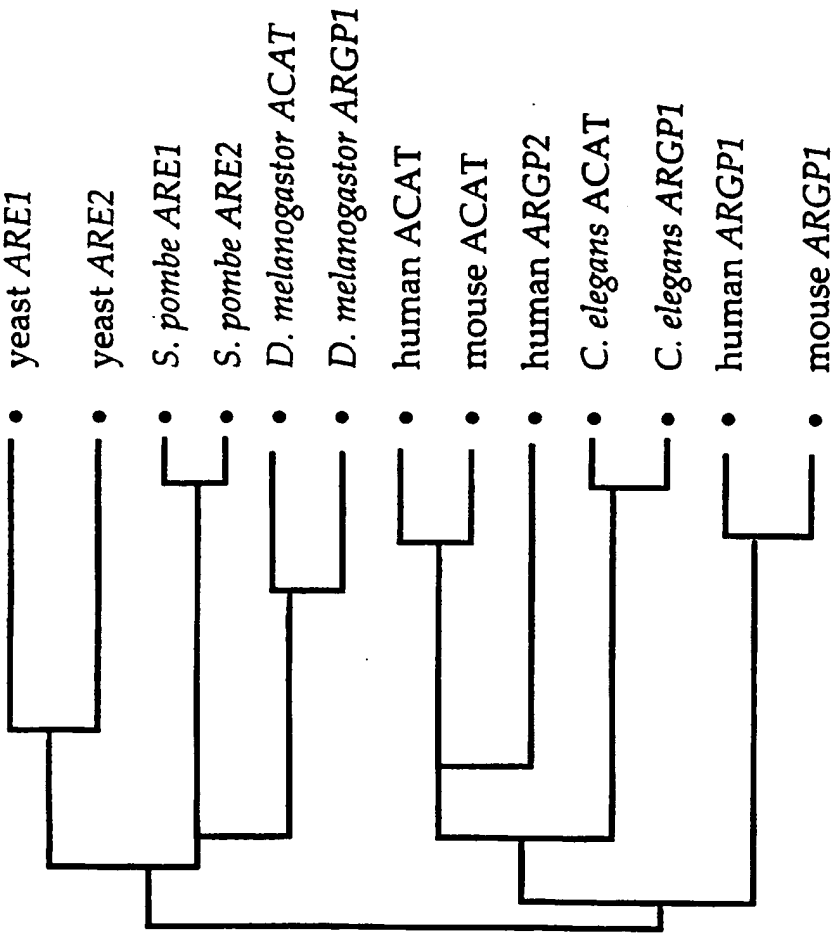

yeast ARE1
yeast ARE2
S. pombe ARE1
S. pombe ARE2
D. melanogastor ACAT
D. melanogastor ARGP1
human ACAT
mouse ACAT
human ARGP2
C. elegans ACAT
C. elegans ARGP1
human ARGP1
mouse ARGP1

FIG. 13

FIG. 14

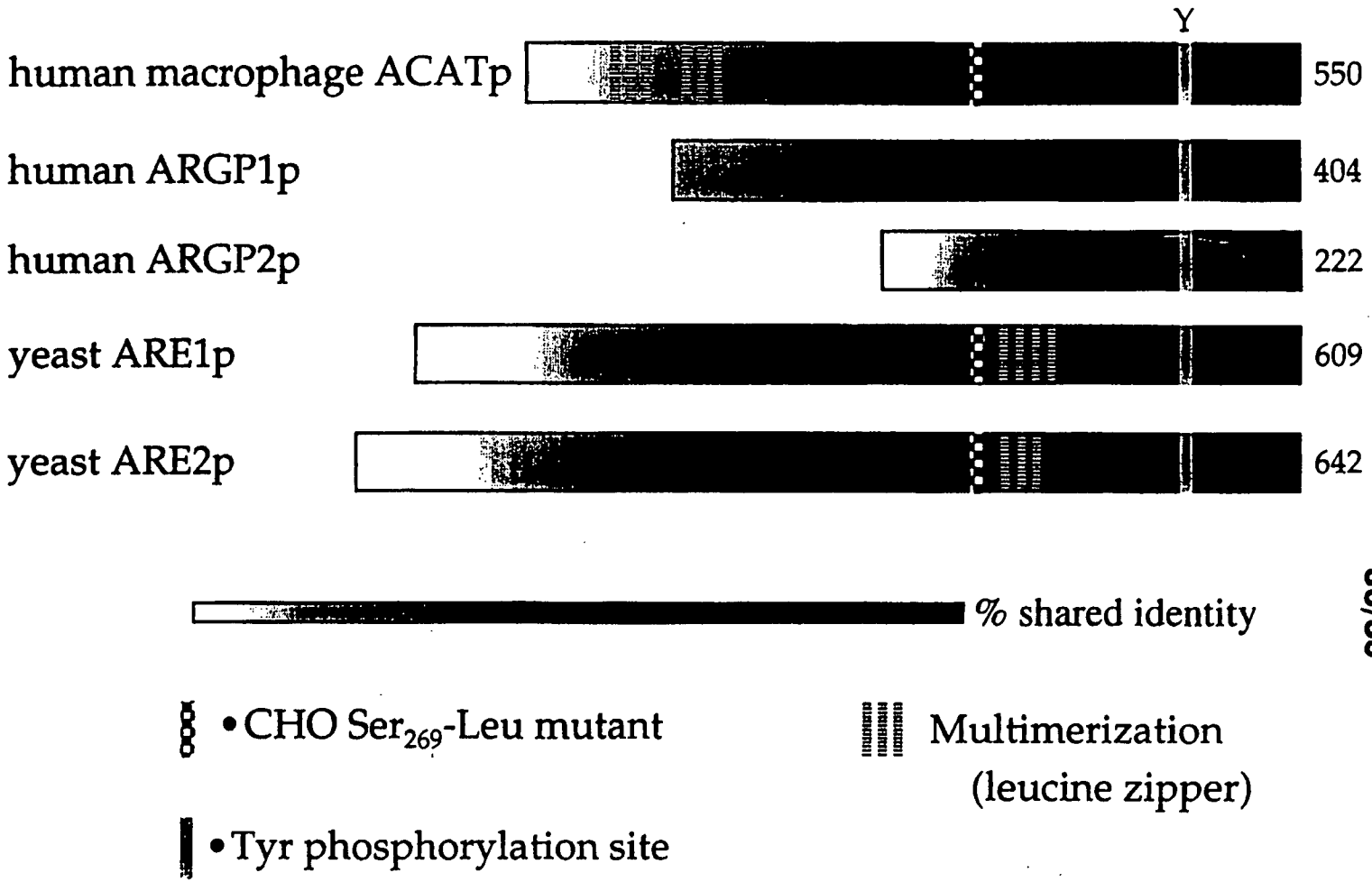

Y
human macrophage ACATp
550
human ARGP1p
404
human ARGP2p
222
yeast ARE1p
609
yeast ARE2p
642
% shared identity
• CHO Ser269-Leu mutant
Multimerization
(leucine zipper)
• Tyr phosphorylation site

FIG. 15A

```
   1 ATG AGC AAC TAC CGT GGC ATC CTG AAC TGG TGT GTG GTG ATG CTG ATC TTG AGC AAT GCC CGG TTA TTT CTG GAG  75
   1 M   S   N   Y   R   G   I   L   N   W   C   V   V   M   L   I   L   S   N   A   R   L   F   L   E  25

  76 AAC CTC ATC AAG TAT GGC ATC CTG GTG GAC CCC ATC CAG GTG GTT TCT CTG TTC CTG AAG GAT CCC CAT AGC TGG 150
  26 N   L   I   K   Y   G   I   L   V   D   P   I   Q   V   V   S   L   F   L   K   D   P   H   S   W  50

 151 CCC GCC CCA TGC CTG GTT ATT GCG GCC AAT GTC TTT GCT GTG GCT GCA TTC CAG GTT GAG AAG CGC CTG GCG GTG 225
  51 P   A   P   C   L   V   I   A   A   N   V   F   A   V   A   A   F   Q   V   E   K   R   L   A   V  75

 226 GGT GCC CTG ACG GAG CAG GCG GGA CTG CTG CTG CAC GTA GCC AAC CTG GCC ACC ATT CTG TGT TTC CCA GCG GCT 300
  76 G   A   L   T   E   Q   A   G   L   L   L   H   V   A   N   L   A   T   I   L   C   F   P   A   A 100

 301 GTG GTC TTA CTG GTT GAG TCT ATC ACT CCA GTG GGC TCC CTG CTG GCG CTG ATG GCG CAC ACC ATC CTC TTC CTC 375
 101 V   V   L   L   V   E   S   I   T   P   V   G   S   L   L   A   L   M   A   H   T   I   L   F   L 125

 376 AAG CCC TTC TCC TAC CGC GAC GTC AAC TCA TGG TGC CGC AGG GCC AGG GCC AAG GCT GCC TCT GCA GGG AAG AAG 450
 126 K   P   F   S   Y   R   D   V   N   S   W   C   R   R   A   R   A   K   A   A   S   A   G   K   K 150

 451 GCC AGC AGT GCT GCT GCC CCG CAC ACC GTG AGC TAC CCG GAC AAT CTG ACC TAC CGC GAT CTC TAC TAC TTC CTC 525
 151 A   S   S   A   A   A   P   H   T   V   S   Y   P   D   N   L   T   Y   R   D   L   Y   Y   F   L 175

 526 TTC GCC CCC ACC TTG TGC TAC GAG CTC AAC TTT CCC CGC TCT CCC CGC ATC CGG AAG CGC TTT CTG CTG CGA CGG 600
 176 F   A   P   T   L   C   Y   E   L   N   F   P   R   S   P   R   I   R   K   R   F   L   L   R   R 200

 601 ATC CTT GAG ATG CTG TTC TTC ACC CAG CTC CAG GTG GGG CTG ATC CAG CAG TGG ATG GTC CCC ACC ATC CAG AAC 675
 201 I   L   E   M   L   F   F   T   Q   L   Q   V   G   L   I   Q   Q   W   M   V   P   T   I   Q   N 225

 676 TCC ATG AAG CCC TTC AAG GAC ATG GAC TAC TCA CGC ATC ATC GAG CGC CTC CTG AAG CTG GCG GTC CCC AAT CAC 750
 226 S   M   K   P   F   K   D   M   D   Y   S   R   I   I   E   R   L   L   K   L   A   V   P   N   H 250
```

FIG. 15B

```
751 CTC ATC TGG CTC ATC TTC TTC TAC TGG CTC TTC CAC TCC TGC CTG AAT GCC GTG GCT GAG CTC ATG CAG TTT GGA  825
251 L   I   W   L   I   F   F   Y   W   L   F   H   S   C   L   N   A   V   A   E   L   M   Q   F   G  275

826 GAC CGG GAG TTC TAC CGG GAC TGG TGG AAC TCC GAG TCT GTC ACC TAC TTC TGG CAG AAC TGG AAC ATC CCT GTG  900
276 D   R   E   F   Y   R   D   W   W   N   S   E   S   V   T   Y   F   W   Q   N   W   N   I   P   V  300

901 CAC AAG TGG TGC ATC AGA CAC TTC TAC AAG CCC ATG CTT CGA CGG GGC AGC AGC AAG TGG ATG GCC AGG ACA GGG  975
301 H   K   W   C   I   R   H   F   Y   K   P   M   L   R   R   G   S   S   K   W   M   A   R   T   G  325

976 GTG TTC CTG GCC TCG GCT TTC TTC CAC GAG TAC CTG GTG AGC GTC CCT CTG CGA ATG TTC CGC CTC TGG GCT TTC 1050
326 V   F   L   A   S   A   F   F   H   E   Y   L   V   S   V   P   L   R   M   F   R   L   W   A   F  350

1051 ACG GGC ATG ATG GCT CAG ATC CCA CTG GCC TGG TTC GTG GGC CGC TTT TTC CAG GGC AAC TAT GGC AAC GCA GCT 1125
351  T   G   M   M   A   Q   I   P   L   A   W   F   V   G   R   F   F   Q   G   N   Y   G   N   A   A  375

1126 GTG TGG CTG TCG CTC ATC ATC GGA CAG CCA ATA GCC GTC CTC ATG TAC GTC CAC GAC TAC TAC GTG CTC AAC TAT 1200
376  V   W   L   S   L   I   I   G   Q   P   I   A   V   L   M   Y   V   H   D   Y   Y   V   L   N   Y  400

1201 GAG GCC CCA GCG GCA GAG GCC TGA gctgcacctgagggcctggcttctcactgccacctcaaacccgctgccagagcccacctctcctccta 1292
401  E   A   P   A   A   E   A   •                                                                    408

1293 ggcctcgagtgctggggatgggcctggctgcacagcatcctcctctggtcccagggaggcctctctgccctatggggctctgtcctgcacccctcaggga 1392

1393 tggcgacagcaggccagacacagtctgatgccagctgggagtcttgctgaccctgccccgggtccgagggtgtcaataaagtgctgtccagtgggagaaa 1492

1493 aaaaaaaaaaaaaaaaattctgcggccgc                                                                        1521
```

FIG. 16

```
   1 ATG TTT ACC CCA GCC CTG GGA TGT GTG TTC TAT GCC TGC TTC ATC GTG GCC CGC TTC TGT GTT CCT GTC TTT GCC   75
   1 M   F   T   P   A   L   G   C   V   F   Y   A   C   F   I   V   A   R   F   C   V   P   V   F   A    25

  76 AAC ATG AGC CGA GAG CCC TTC AGC ACC CGT GCC TTG GTG TTT TTT ATC CTG CAT GCC ACG TTG CCA GGC ATT TTC  150
  26 N   M   S   R   E   P   F   S   T   R   A   L   V   F   F   I   L   H   A   T   L   P   G   I   F    50

 151 ATG CTG CTG CTC ATC TTC TTT GCC TTC CTC CAT TGC TGG CTC AAC GCC TTT GCC GAG ATG CTA CGA TTT GGA GAC  225
  51 M   L   L   L   I   F   F   A   F   L   H   C   W   L   N   A   F   A   E   M   L   R   F   G   D    75

 226 AGG ATG TTC TAC CGG GAC TGG TGG AAC TCA ACG TCC TTC TCC AAC TAC TAC CGC ACT TGG AAC GTG GTG GTC CAT  300
  76 R   M   F   Y   R   D   W   W   N   S   T   S   F   S   N   Y   Y   R   T   W   N   V   V   V   H   100

 301 GAC TGG CTG TAC AGC TAC GTG TAT CAG GAT GGG CTG CGG CTC CTT GGT GCC CGG GCC CGA GGG GTA GCC ATG CTG  375
 101 D   W   L   Y   S   Y   V   Y   Q   D   G   L   R   L   L   G   A   R   A   R   G   V   A   M   L   125

 376 GGT GTG TTT CTG GTC TCC GCA GTG GCC CAC GAG TAT ATC TTC TGC TTC GTC CTG GGG TTC TTC TAT CCC GTC ATG  450
 126 G   V   F   L   V   S   A   V   A   H   E   Y   I   F   C   F   V   L   G   F   F   Y   P   V   M   150

 451 CTG ATA CTC TTC CTT GTC ATT GAA GGA ATG TTG AAC TTC ATG ATG CAT GAC CAG CGC ACC GGC CCG GCA TGG AAC  525
 151 L   I   L   F   L   V   I   E   G   M   L   N   F   M   M   H   D   Q   R   T   G   P   A   W   N   175

 526 GTG CTG ATG TGG ACC ATG CTG TTT CTA GGC CAG GGA ATC CAG GTC AGC CTG TAC TGC CAG GAG TGG TAC GCA CGG  600
 176 V   L   M   W   T   M   L   F   L   G   Q   G   I   Q   V   S   L   Y   C   Q   E   W   Y   A   R   200

 601 CGG CAC TGC CCC TTA CCC CAG GCA ACT TTC TGG GGG CTG GTG ACA CCT CGA TCT TGG TCC TGC CAT ACC TAG aggt  676
 201 R   H   C   P   L   P   Q   A   T   F   W   G   L   V   T   P   R   S   W   S   C   H   T   *       224

 677 cgggacagacgacgctacctgcccagacaccaccaagttctctgcctgcaaaacctggggaccaggactccctgtctgcattccccaaatttggctctga  776

 777 gtcgaggcaacctgcacacaagacccccacccaaggaatgtgcaaggactgagatctgcagacttgtgggtaactgatcacagacctcagcatgggggtg  876

 877 accagggtgactcttcaatccctatccccatgggctgggtacaggatatcctcctaccccatgactgtcttagggagacttggggtaccttatggatttg  976

 977 atgaatgtgggggaactcagaggaactggggccaccaaggttggaaaagggtttggttcttgactttgtattccttccaatacagcaataaactttgtct 1076

1077 cccttt                                                                                              1082
```

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/09460

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07H 21/04; C12N 15/11; C12P 21/02
US CL :536/23.2, 23.5; 435/70.1, 320.1, 325
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.2, 23.5; 435/70.1, 320.1, 325

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, BIOSIS, CA, DERWENT
search terms: acylcoenzyme A: cholesterol acyltransferase

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	Expressed Sequence Tag (EST) database Accession Numbers R99213 (13 September 1995), R99214 (13 September 1995), H27149 (12 July 1995), N76754 (02 April 1996), H45923 (31 July 1995), H45924 (31 July 1995), H24791 (07 July 1995), Z39933 (21 September 1995), T79408 (15 March 1995), R48474 (18 May 1995), T79494 (15 March 1995), R48475 (18 May 1995), M79086 (26 May 1992), and W10786 (26 April 1996).	1-12 and 33 ------- 13-32
Y	US 5,484,727 A (CHANG et al.) 16 January 1996, cols. 1-5.	13-32

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 19 AUGUST 1997	Date of mailing of the international search report 29 AUG 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer KENNETH R. HORLICK Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/09460

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-33

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/09460

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s)1-33, drawn to acylcoenzyme A: cholesterol acyltransferase II and III nucleic acids and enzymes, and a first method of use comprising recombinant expression.
Group II, claim(s) 34-39, drawn to a second method of use comprising detecting a defect in esterification of sterol in a subject.
Group III, claim(s) 40, drawn to third method of use comprising treating a subject having an imbalance in sterol levels.
Group IV, claims 41-44, drawn to a fourth method of use comprising inhibiting wildtype acylcoenzyme A: cholesterol acyltransferase II or III in a subject.
Group V, claim 45, drawn to a fifth method of use comprising identifying a chemical compound which is capable of inhibiting acylcoenzyme A: cholesterol acyltransferase II or III in a subject.
Group VI, claims 46-48, drawn to an undefined pharmaceutical compound and methods of its use in treating a subject.
Group VII, claim 49, drawn to a transgenic, nonhuman mammal.

The inventions listed as Groups I-VII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: PCT Rule 13.2 only provides for grouping together of a first product and a first method of use, rather than for multiple uses. Further, the compound and uses of group VI and the transgenic mammal of group VII lack the same or corresponding special technical feature as the other groups, which relate specifically to the acylcoenzyme A:choloesterol acyltransferase II or III sequence.